CHROMOSOMA

ZEITSCHRIFT FÜR
ZELLKERN- UND CHROMOSOMENFORSCHUNG

1. BAND

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ZEITSCHRIFT FÜR ZELLKERN- UND CHROMOSOMENFORSCHUNG

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DIE ENTSTEHUNG DER POLYPLOIDEN SOMAKERNE DER HETEROPTEREN DURCH CHROMOSOMENTEILUNG OHNE KERNTEILUNG.

Von

LOTHAR GEITLER, Wien.

Mit 11 Textabbildungen (62 Einzelbildern).

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Inhaltsübersicht.

I. Einleitung und Fragestellung.

An einer Heteroptere, dem Wasserläufer *Gerris lateralis* var. *costae*, ließ sich feststellen, daß die Kerne somatischer Gewebe in bestimmter Weise polyploid sind (GEITLER 1937, 1938a). Die größten Kerne, die in der Speicheldrüse vorkommen, sind 1024- oder 2048-ploid, die Kerne der als Oenocyten angesprochenen Zellen 128-ploid, der MALPIGHIschen Gefäße 32-ploid usw. Diese Feststellungen werden dadurch ermöglicht, daß die Autosomen und das X-Chromosom in den Ruhekernen im allgemeinen distinkt erhalten bleiben; in mäßig polyploiden Ruhekernen lassen sich die Chromosomen meist unmittelbar auszählen; im hochpolyploiden kann meist die Anzahl der X-Chromosomen festgesetzt werden (im ♂ entspricht dem diploiden Autosomensatz 20 ein X-Chromosom, im ♀ entsprechen ihm zwei X-Chromosomen). Diese Tatsachen sind durch die eindeutigen morphologischen Verhältnisse sowie durch die in niedriger polyploiden Kernen manchmal ablaufenden Mitosen endgültig bewiesen.

Die Fragestellungen, die sich hieran anschließen, sind folgende: 1. wieweit ist dieser Typus des Kernbaus (und damit der Gewebedifferenzierung) in gleicher oder ähnlicher Ausbildung verbreitet? 2. welche Beziehungen bestehen zwischen diesen definierten Kernstrukturen und der Funktion? 3. Wie entstehen die polyploiden Kerne?

Der Beantwortung der dritten Frage, die zunächst am dringendsten erscheint, sind die folgenden Mitteilungen hauptsächlich gewidmet[1];

[1] Eine kurze Mitteilung erschien bereits in den „Naturwissenschaften" (GEITLER 1938b).

dabei wird auch der zweitgenannte Fragenkomplex gestreift werden. Der erste Programmpunkt wird, soweit es sich um die Verhältnisse anderer Organismengruppen handelt, im allgemeinen Teil kurz erörtert werden; für die Heteropteren selbst liegen bereits weitere Beobachtungen an *Gerris lacustris*, einer unbestimmten Gerridide, an *Velia currens* (Veliide) und *Macrotylus quadrilineatus* (Capside) vor (GEITLER 1938a): sie zeigen erwartungsgemäß, daß auch bei diesen Arten grundsätzlich der gleiche Kernbau verwirklicht ist. Dies gilt auch für die folgenden seither untersuchten Arten: *Lygus pratensis* und *innotatus* (Capsiden), *Pyrrhocoris apterus* (Pyrrhocoride), *Lygaeus saxatilis* (Lygaeide), *Syromastes marginatus* (Coreide), *Micronecta minutissima* und *Sigara carinata* (Corixiden), *Graphosoma italicum*, *Eurydema dominulus* und *oleraceum*, *Palomena prasina*, *Dolycoris baccarum*, *Carpocoris melanocerus*, *Pentatoma rufipes*, *Eurygaster maurus*, *Picromerus bidens* (Pentatomiden)[1]. Im ganzen sind also mit übereinstimmendem Ergebnis 21 Arten aus 8 Familien untersucht. Im einzelnen zeigen sich allerdings bestimmte Modifikationen, die wahrscheinlich wenigstens zum Teil davon abhängen, ob der X-O- oder der X-Y-Typus verwirklicht ist (vgl. GEITLER (1938b). Die eingehende Schilderung dieser Verhältnisse wird an anderer Stelle erfolgen.

Die folgende Bearbeitung wurde ausschließlich an *Gerris lateralis* vorgenommen, da diese Art infolge der bedeutenden Größe des X-Chromosoms und der Entwicklung nach dem X-O-Typus weitaus am geeignetsten ist. Daß die Ergebnisse auch für die anderen Wanzen gültig sind, ergibt sich aus dem übereinstimmenden Kernbau; außerdem zeigten an einigen der angeführten Arten vorgenommene Stichproben die erwartete Übereinstimmung. (Belege werden in einer späteren Veröffentlichung gebracht werden).

II. Material und Technik.

Um die Entstehung der Polyploidie verfolgen zu können, war es nötig, die bisherigen, fast ausschließlich an der Imago durchgeführten Untersuchungen auf die früheren Entwicklungsstadien auszudehnen. Es war zu erwarten, daß die Larven Stadien der gesuchten Kernentwicklung enthalten würden, so daß von der schwierigen Beschaffung von Eiern bzw. Embryonen abgesehen werden konnte. Eine orientierende Untersuchung bestätigte die Vermutung, daß bestimmte, in der Imago polyploide Kerne in den jüngsten Larven noch diploid sind.

Es wurde daher eine größere Zahl von Larven aller Altersstufen teils zerschnitten in Alkohol-Eisessig fixiert, teils sofort nach Betäubung zu Zupfpräparaten verarbeitet und über Alkohol-Eisessig, Essigkarmin und Alkohol in venezianischen Terpentin übergeführt. Die in Alkohol-Eisessig fixierten Tiere wurden

[1] Herrn Kollegen W. KÜHNELT danke ich auch an dieser Stelle für die Revision bzw. Bestimmung.

in 96%igem Alkohol aufbewahrt und später in Essigkarmin untersucht; es empfiehlt sich, dabei die Überführung von Alkohol in Essigkarmin über Alkohol-Eisessig vorzunehmen, da dadurch das Ausflocken des Karmins vermieden wird und die Färbung besser gelingt. Die beigegebenen Zeichnungen sind nach solchen in Essigkarmin liegenden Geweben angefertigt; infolge der stärkeren Quellung und der größeren Lichtbrechungsunterschiede lassen sie sich optisch leichter analysieren als Präparate in venezianischem Terpentin. Über die Grenzen der Alkohol-Eisessig-Essigkarmin-Technik wurde das Nötige schon in der 1. Mitteilung gesagt (1937); sie ist trotz gewissen Nachteilen für die hier verfolgten Ziele anderen Methoden weit überlegen[1].

Das Material stammt aus einem Almtümpel des Hetzkogelplateaus bei Lunz (Nied.-Donau), wo sich in den Monaten August und September reichlich alle Larvenstadien finden[2].

Gerris lateralis durchläuft fünf Larvenstadien, die im folgenden als I. bis V. Stadium bezeichnet werden (vgl. MITTIS 1937)[3]. Spermatocyten finden sich schon im IV. Stadium, im V. Stadium treten auch Spermien auf. Die Bestimmung des Geschlechts jüngster Larvenstadien erfolgt durch die Analyse des Baus diploider oder tetraploider Kerne, indem festgestellt wird, ob 1 oder 2 bzw. 2 oder 4 X-Chromosomen vorhanden sind. Dabei ist allerdings zu beachten, daß sich häufig, besonders im ♀, zwei X-Chromosomen zu einem Sammelchromozentrum vereinigen können (s. weiter unten); Irrtümer lassen sich aber dadurch ausschalten, daß die Autosomen gezählt werden und daß die *Größe* der X-Chromozentren berücksichtigt wird; zudem finden sich wenigstens in manchen Kernen auch getrennte X-Chromosomen.

III. Untersuchungen.

1. Übersicht.

Die Morphologie und Histologie der Organe von *Gerris* ist, wie bei den meisten Wanzen, nicht bearbeitet. Es mußte daher die Untersuchung auf bestimmte, leicht identifizierbare und auch in den jüngsten Larvenstadien sicher auffindbare, gut präparierbare Organe bzw. Gewebe beschränkt werden (vgl. für das folgende auch die Schilderung in den früheren Mitteilungen 1937 und 1938a). Als geeignet für die Verfolgung der Gesamtentwicklung vom I. Stadium an erwiesen sich die Kerne der MALPIGHIschen Gefäße; sie sind im I. Stadium diploid und

[1] Die Güte der Alkohol-Eisessig-Fixierung kommt am besten zur Geltung, wenn unmittelbar aus dem Fixierungsgemisch in Essigkarmin übertragen wird; Zwischenschaltung von Alkohol wirkt schon ungünstig; im besonderen ist die Einwirkung von Wasser zu vermeiden. Für die Mikrotomtechnik ist die Alkohol-Eisessig-Fixierung wenig geeignet, da sie keine stabilen Strukturen liefert; diese Erfahrungstatsache zeigte sich auch an den Schnittserien, die zum Zweck topographischer Untersuchungen der Organe angefertigt wurden.

[2] Für die Beschaffung bin ich dem Laboranten der Biologischen Station Lunz, Herrn SEPP AIGNER, zu Dank verpflichtet.

[3] Herrn v. MITTIS danke ich auch an dieser Stelle für seine Hilfe bei der Bestimmung der Stadien.

werden allmählich 32-ploid (in den distalen Teilen geht das Kernwachstum nicht so weit, die imaginalen MALPIGHIschen Gefäße sind daher in Abschnitte mit verschieden großen Kernen differenziert). Die feineren Vorgänge bei der Chromosomenvermehrung werden besonders in den Kernen der Hodensepten deutlich, da sie locker gebaut und stark abgeflacht sind; zudem setzt vom IV. Stadium an eine schnelle Entwicklung vom diploiden zum 16- (oder manchmal auch 32-) ploiden Zustand in der Imago ein, so daß man leicht „Teilungsstadien" auffinden kann.

An diesen Kernen ließ sich die Polyploidisierung schrittweise verfolgen. Einzelne der bezeichnenden Phasen des Ablaufs wurden außerdem beobachtet in der bindegewebigen Hülle des Hodens, im Follikelepithel der Ovarien, in der Speicheldrüse, im Fettkörper und in den als Oenocyten angesehenen Zellen, schließlich im Mitteldarmepithel, wo sich die Polyploidisierung bei der Entstehung der holokrinen Drüsenzellen aus den diploiden Erneuerungszellen abspielt. Die Beispiele genügen, um zu zeigen, daß die Polyploidisierung in allen Fällen *in grundsätzlich gleicher Weise erfolgt.*

2. Kerne der Hodensepten.

An Hoden älterer Larven läßt sich eine Reihe von Entwicklungsphasen der Septenkerne beobachten, welche die wesentlichen Aufschlüsse über die Polyploidisierung bietet. Die Polyploidisierung ist im allgemeinen bereits abgeschlossen, wenn die Spermatocyten der zugehörigen Cyste sich im späten Pachytänstadium befinden. Die ältesten, stark abgeflachten Kerne sind 16-ploid, besitzen also 8 heterochromatische, mehr oder minder zentral gelegene X-Chromosmen und 160 lockerer gebaute und schwächer färbbare Autosomen (1937, Abb. 3f—l; selten kommen auch 32-ploide Kerne vor — Fig. h). Einige der X-Chromosomen können zu je zweien zu Sammelchromozentren vereinigt sein (unsere Abb. 1g), was aber selten ist.

Septenkerne der jüngeren Teile des Hodens besitzen 4, 2 oder 1 X-Chromosom (Abb. 2) und die dementsprechende Zahl von Autosomen. Die Autosomen dieser Kerne sind kompakter, sie liegen dichter und die Kerne selbst sind noch nicht so stark abgeflacht wie die fertiggestellten; Abb. 1f zeigt einen bereits 16-ploiden Kern bald nach seiner Entstehung.

Unter den jungen Kernen findet man solche, die einen vom gewohnten Aussehen auffallend abweichenden Bau besitzen. Statt einzelner, mehr oder weniger abgerundeter oder unregelmäßig umrissener, chromozentrenartiger Chromosomen sind *Zweiergruppen* stäbchenförmiger Chromosomen vorhanden (Abb. 2b, d, e, j, k). Die X-Chromosomen unterscheiden sich von den Autosomen abgesehen von ihrer Größe durch die dichtere Beschaffenheit, stärkere Färbbarkeit und den engeren Zusammenhalt ihrer „Partner". Die „Partner" der Autosomen hängen häufig an einem oder an beiden Enden zusammen. Die Chromosomen sind im übrigen wie in einem Ruhekern verteilt.

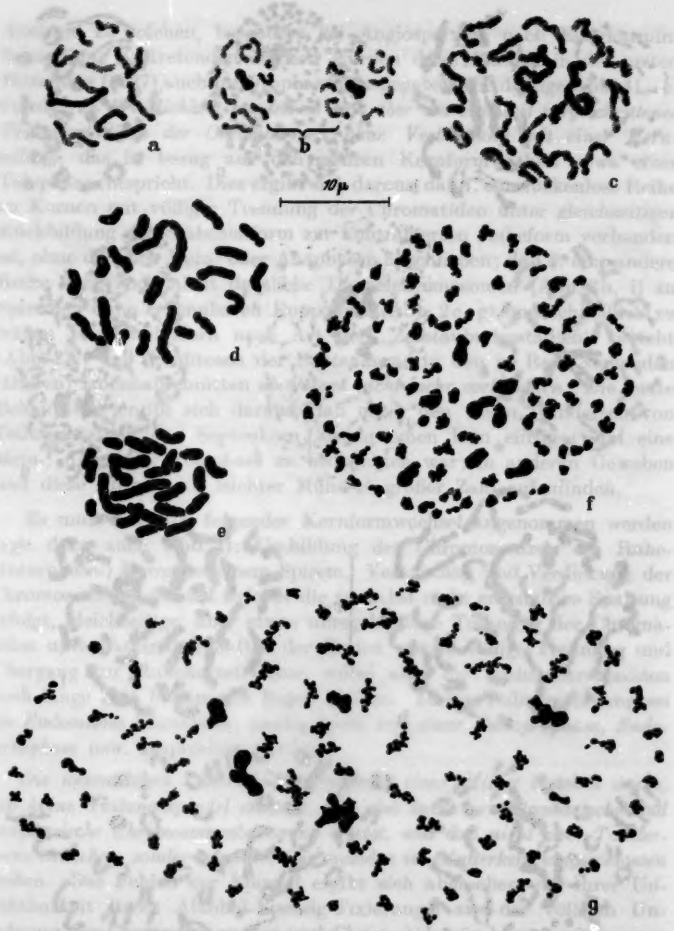

Abb. 1a—g. a Diploide Prophase und b Telophase im Bindegewebe (Männchen). c—e diploide mittlere Prophase bis frühe Metaphase (c, e Weibchen, d Männchen); in c sind die beiden X-Chromosomen von den Autosomen noch unterschieden); f 16-ploider Hodenseptenkern eben nach seiner Entstehung (8 X-Chromosomen, Autosomen noch relativ kompakt und dicht liegend); g fertiggestellter 16-ploider Hodenseptenkern (2 einfache X-Chromosomen und 3 aus je 2 X-Chromosomen bestehende Chromozentren; Autosomen aufgelockert und locker liegend).

Dieses Stadium könnte ohne weitere Kenntnis für eine späte Prophase mit sehr deutlich getrennten Chromatiden gehalten werden. In

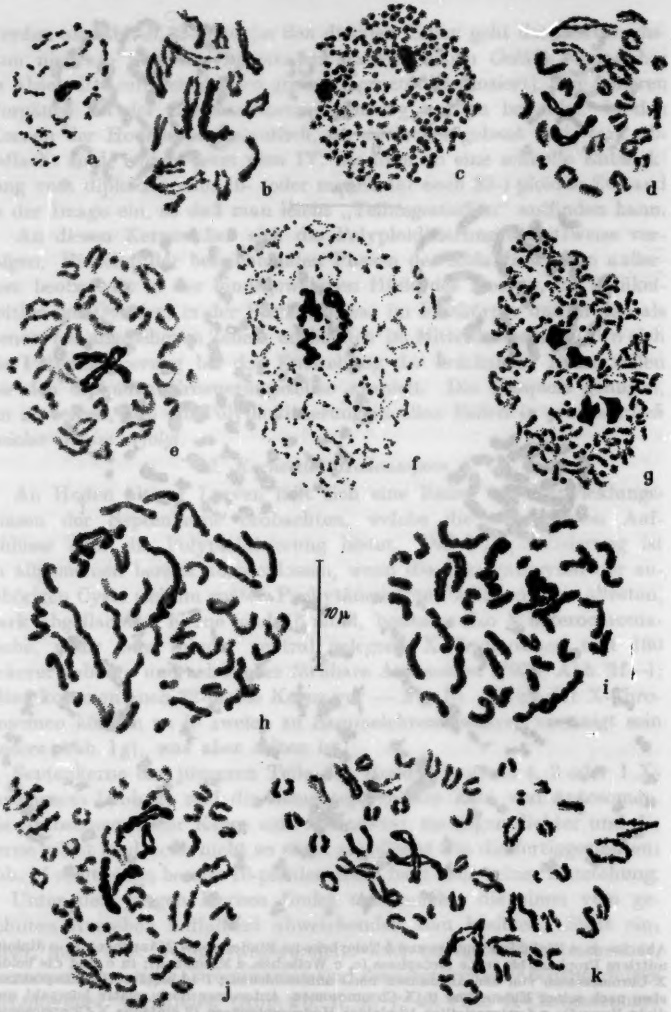

Abb. 2 a—k. Entwicklung der Septenkerne im Hoden (V. Stadium). a diploider Ausgangskern, wenig abgeflacht; b Endotelophase eines diploiden Kerns: ein gespaltenes X-Chromosom und 20 Doppelautosomen (nicht alle gezeichnet!); c Endoprophase eines tetraploiden Kerns (frühes Endospirem). d, e tetraploide Kerne in Endotelophase. f, g sehr frühe und frühe Endoprophase oktoploider Kerne; h ebenso, etwas später (von den 4 X-Chromosomen liegen je 2 — in der vorhergehenden Endomitose auseinander entstandene — nebeneinander); i ebenso, Endometa-Anaphase (Spalt in einigen Autosomen eben sichtbar geworden, je 2 X-Chromosomen beisammen liegend); j oktoploider Kern in Endotelophase; k ebenso, etwas gedrückt: 160 Autosomen, noch paarweise einander genähert, 8 X-Chromosomen, je 2 Schwesterchromosomen noch dicht beisammen, die Enkelchromosomen einander genähert.

Analogie zu solchen, besonders bei Angiospermen nach Essigkarminbehandlung auftretenden Bildern wurden diese Stadien in der ersten Mitteilung (1937) auch für Prophasen ausgegeben (die dortige Abb. 4l—n, 6c, d). *In Wirklichkeit handelt es sich aber um ein weit vorgeschrittenes Teilungsstadium der Chromosomen ohne Verbindung mit einer Kernteilung,* das in bezug auf den ganzen Kernformwechsel etwa einer Telophase entspricht. Dies ergibt sich daraus, daß 1. eine lückenlose Reihe zu Kernen mit völliger Trennung der Chromatiden unter gleichzeitiger Rückbildung der Stäbchenform zur kontrahierten Ruheform vorhanden ist, ohne daß sich Meta- oder Anaphasen anschließen; daß 2. eine andere Reihe über noch nicht deutliche Doppelchromosomen (Abb. 2h, i) zu Spiremen ohne erkennbaren Doppelbau (Abb. 2c, g) und schließlich zu frühen Prophasebildern nach Art der „Zerstäubungsstadien" besteht (Abb. 2f); daß 3. Mitosen der Septenkerne in den in Rede stehenden (älteren) Hodenabschnitten *überhaupt nicht mehr vorkommen.* Die letzte Behauptung ergibt sich daraus, daß unter den vielen Hunderten von Teilungsstadien der Septenkern-Chromosomen kein einziges Mal eine Meta-, Ana- oder Telophase zu beobachten war; in anderen Geweben sind diese Phasen mit leichter Mühe in großer Zahl aufzufinden.

Es muß demnach folgender Kernformwechsel angenommen werden (vgl. dazu auch Abb. 3): Umbildung der Chromozentren des Ruhe- (Interphase-) Kerns zu einem Spirem. Verkürzung und Verdickung der Chromosomen, während welcher die zunächst nicht erkennbare Spaltung erfolgt, gleichzeitige, aber etwas unregelmäßige Trennung der Chromatiden unter Aneinanderhaften der Enden, später völlige Trennung und Übergang zur Ruhekernstruktur, wobei aber die Tochterchromatiden noch lange Zeit beisammen liegen bleiben. Dieser Teilungsvorgang sei als *Endomitose* bezeichnet; analog kann von einer *Endoprophase, Endometaphase* usw. gesprochen werden.

Die wesentlichen Unterschiede gegenüber einer Mitose bestehen darin, daß keine Teilungsspindel entsteht, daß also keine prometaphasische und anaphasische Chromosomenbewegung erfolgt, und daß nicht zwei Tochterkerne entstehen, sondern daß die Chromatiden im Mutterkern eingeschlossen bleiben. Das Fehlen der Spindel ergibt sich abgesehen von ihrer Unsichtbarkeit (trotz Alkohol-Eisessig-Fixierung!) aus der völligen Unordnung der Chromatidenpaare und Chromatiden in bezug auf eine anzunehmende Spindelachse.

Der Anfang des Teilungsablaufs gleicht in allen wesentlichen einer gewöhnlichen Prophase bis zu dem Zeitpunkt, wo bei dieser die Spindel in Wirksamkeit tritt. Das Spirem einer gewöhnlichen Mitose erscheint bei *Gerris* immer ungespalten (Abb. 1a, c). Frühe bis mittlere Prophasen lassen sich daher nicht ohne weiteres von Endoprophasen unterscheiden; eine sichere Entscheidung ist eigentlich nur dann möglich,

wenn für eine bestimmte Gewebeart das alleinige Vorkommen der einen oder anderen Teilungsart festgestellt ist. Erst in den späten Prophasen bzw. Prometaphasestadien treten deutliche Unterschiede hervor: im Fall der Endomitose unterbleibt die Bewegung der Chromosomen in den Äquator, es unterbleibt die starke mitotische Verkürzung und Verdickung, die in der Metaphase ihren Höhepunkt erreicht, und es unterbleibt

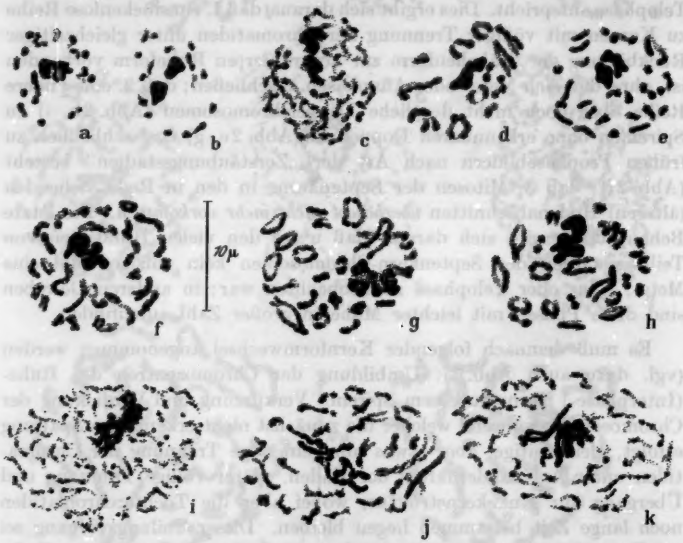

Abb. 3a—k. Entwicklung der Kerne in den MALPIGHIschen Gefäßen des Weibchens. a, b I. Stadium, diploide Kerne (in a sind die beiden X-Chromosomen vereinigt); c—k II. Stadium: c Endoprophase eines diploiden Kerns; d Endotelophase; e Interphasekern; f—h Endotelophase in tetraploiden Kernen. i—k tetraploide Kerne aus einem anderen MALPIGHIschen Gefäß; lockerere Struktur, besonders die Spaltung der X-Chromosomen deutlich; i Endoprophase; j Endoanaphase; k Interphase.

die völlige Angleichung der Beschaffenheit der heterochromatischen X-Chromosomen an die Autosomen. Die X-Chromosomen bleiben also dauernd kompakter, sie geben die chromozentrenartige Ausbildung nicht wie die Autosomen ganz auf. Die heterochromatische Beschaffenheit hat zur Folge, daß die X-Chromatiden in der Endoanaphase später als die der Autosomen getrennt sichtbar werden, und daß die Tochter-X-Chromosomen auch noch nach der völligen Trennung der Autosomenchromatiden vereinigt bleiben, also ein aus zwei X-Chromosomen bestehendes Sammelchromozentrum bilden. Solche Doppel-X-Bildungen sind im übrigen keine Eigentümlichkeit der Endomitose, sondern finden sich sehr häufig auch in diploiden Kernen des Weibchens (z. B. Abb. 3a);

ontogenetisch sind diese Bildungen allerdings verschieden, indem im 1. Fall Schwesterchromosomen, im anderen homologe Chromosomen vereinigt sind[1].

Das Verhalten der Nukleolen während der Endomitose läßt sich an Essigkarminpräparaten nicht völlig klären. Es ist jedoch deutlich, daß die Nukleolen nicht unverändert bleiben, sondern so wie in der Mitose in den mittleren Stadien weitgehend abgebaut werden (wenn sie nicht überhaupt verschwinden)[2]. Sobald die Chromatiden deutlich getrennt sind, aber noch mit ihren Enden aneinander hängen, sind die Nukleolen bereits wiederhergestellt. Auch hieraus (wie aus der langen Dauer dieses Stadiums) wird es deutlich, daß diese Bilder nicht etwa einer Anaphase, sondern einer Telophase entsprechen.

3. *Kerne der* Malpighischen *Gefäße.*

Die Kerne der Malpighischen Gefäße sind im I. Stadium durchwegs diploid: im Männchen sind ein X-Chromosom und 20 Autosomen, im Weibchen sind zwei X-Chromosomen und 20 Autosomen vorhanden. Die beiden X-Chromosomen im Weibchen sind fast immer zu einem Sammelchromozentrum vereinigt (Abb. 3a); nur ausnahmsweise kommen Kerne mit getrennten X-Chromosomen vor (Abb. 3b). Die Autosomen sind unregelmäßig fädig entwickelt. Im II. Stadium findet man tetraploide, im III. oktoploide, im IV. 16-ploide, vom V. Stadium an 32-ploide Kerne (wie erwähnt, nehmen nicht alle Kerne der Malpighischen Gefäße an dieser Entwicklung teil).

Der Vorgang der Polyploidisierung ist der gleiche wie im Fall der Hodenseptenkerne (Abb. 3—5). Die Chromosomen sind, besonders in den älteren Stadien, oft ziemlich kompakt und formveränderlich, was vielleicht mit der jeweiligen Funktion der Zellen zusammenhängt. Die Spaltung der X-Chromosomen ist infolge ihres Chromatinreichtums manchmal maskiert. Der Unterschied im Aussehen ruhender Kerne und der in Endomitose befindlichen ist besonders in hochpolyploiden Kernen sehr auffallend (Abb. 4, 5).

Mitosen wurden niemals gesehen. Dagegen wurden einmal in einem V. Stadium einige Kerne beobachtet, die eine nicht völlig zu Ende

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[1] Über die Vereinigung oder Trennung der X-Chromosomen entscheidet zum Teil auch die relative Menge des Kernsafts, was sich aus dem Vergleich kleiner und großer diploider Ganglienkerne ergibt: in den kleinen sind zwei getrennte X-Chromosomen sehr selten, in den großen die Regel (Abb. 10c, d).

[2] In der I. meiotischen Anaphase ist ein kleiner Restkörper des Nukleolus erhalten. — *Gerris lateralis* besitzt, wie sich in der I. meiotischen Prophase feststellen läßt, ein Sat-Chromosomenpaar. In den polyploiden Kernen sind die primären Nukleolen zu einem oder zu wenigen entsprechend großen Sammelnukleolen vereinigt.

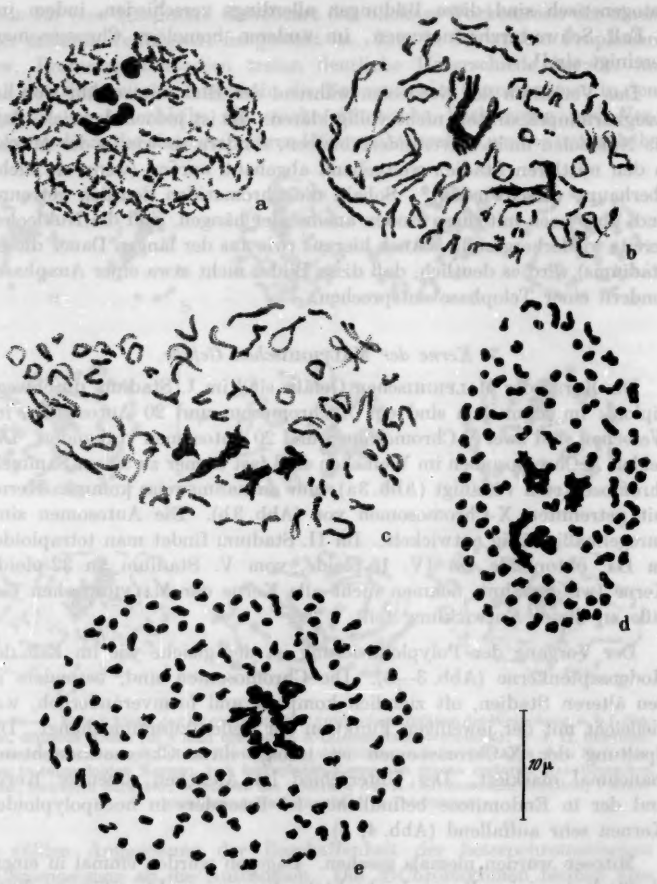

Abb. 4a—e. Spätere Entwicklungsstadien der Kerne in den MALPIGHIschen Gefäßen des Männchens (IV. Stadium). a Endoprophase in einem oktoploiden Kern; b, c Endotelophase in oktoploiden Kernen; d, e 16-ploide Interphasekerne.

geführte Amitose durchgemacht zu haben schienen; eine sichere Entscheidung war nicht möglich[1].

[1] Sie ist für das hier behandelte Thema zunächst ohne Bedeutung, darüber hinaus allerdings von Wichtigkeit (ILSE FISCHER, RIES; vgl. auch den allgemeinen Teil).

4. Endomitosen in Kernen anderer Gewebe.

Die größten Kerne der Speicheldrüse, die in der Imago mindestens 1024-ploid werde, sind im I. Stadium bereits 64-ploid. Infolge des komplizierten Baus der Speicheldrüse ist eine sichere Identifizierung der

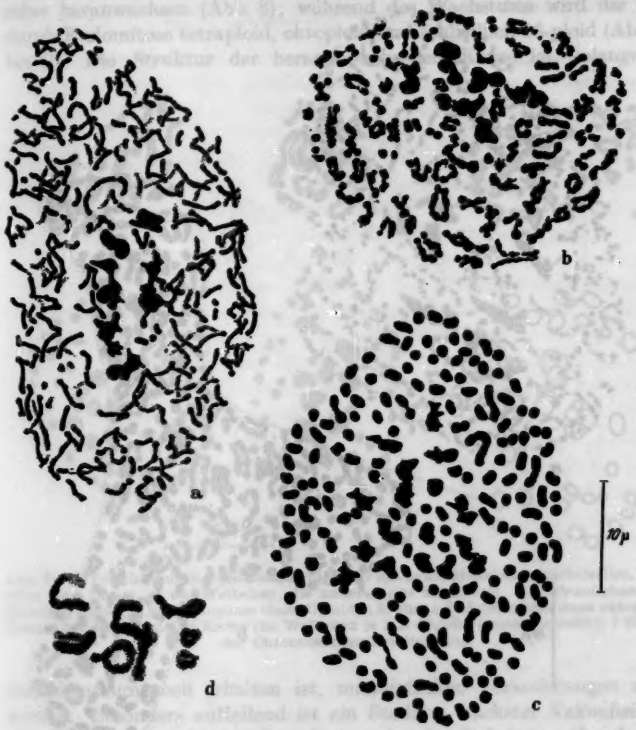

Abb. 5a—d. Kernentwicklung in den MALPIGHIschen Gefäßen, V. Stadium. Männchen. a mittlere Endoprophase in einem 16-ploiden Kern, b Endotelophase in einem 16-ploiden Kern; c 32-ploider Ruhekern mit 16 getrennten X-Chromosomen; d X-Chromosomengruppe aus einem etwas jüngerem Kern wie der in c dargestellte: 16 X-Chromosomen, je 2 noch zusammenhängend und nicht chromozentrenartig kondensiert.

einzelnen Abschnitte und der entsprechenden Kerne allerdings schwierig. Die Kerne sind schon im I. Stadium der Zellform entsprechend stark abgeflacht, jedoch noch nicht oder kaum gelappt. Die Lappung beginnt im II. Stadium sichtbar zu werden, im III. Stadium wird bereits die für die imaginale Speicheldrüse so bezeichnende Verästelung deutlich (vgl. 1938a, Abb. 3). Eine Endoprophase und eine Endotelophase

stellt Abb. 6 dar; der Ablauf ist durchaus der gleiche wie in den oben geschilderten Fällen.

Ebenfalls im I. Stadium schon polyploid sind die Kerne der als Oenocyten bezeichneten Zellen, die in der Imago bis 128-ploid werden.

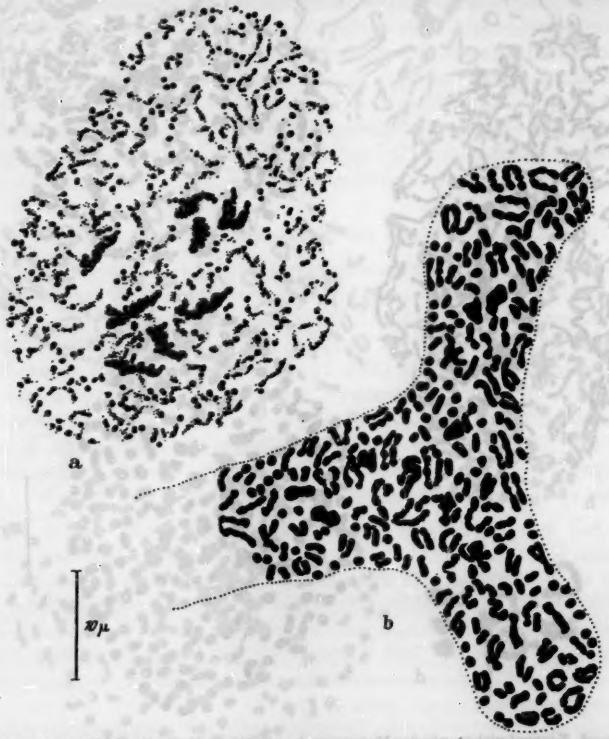

Abb. 6a und b. a 16-ploider Kern aus der Speicheldrüse in früher Endoprophase (I. Stadium, Männchen); b Teil eines wahrscheinlich 256-ploiden Kerns aus der Speicheldrüse in Endotelophase (IV. Stadium, Männchen).

Auch in ihnen laufen Endomitosen ab. Das gleiche gilt für die im 1. Abschnitt aufgezählten anderen Kernarten.

Eine gesonderte Besprechung bedürfen die Kerne der Drüsenzellen des Mitteldarmepithels. Diese im Zusammenhang mit der Verdauung resorbierenden, sezernierenden und schließlich zugrunde gehenden Zellen enthalten vom Beginn ihrer Funktion an bzw. schon etwas früher 16-ploide Kerne; im Männchen sind die acht X-Chromosomen getrennt

oder teilweise zu je zweien vereinigt, im Weibchen werden von den 16 x-Chromosomen fast immer acht Zweierchromozentren gebildet (Abb. 7d, e). Die Polyploidisierung erfolgt jedesmal bei der Zellerneuerung, indem aus den basalen Nestern diploider Erneuerungszellen einzelne heranwachsen (Abb. 8); während des Wachstums wird der Kern durch Endomitose tetraploid, oktoploid und schließlich 16-ploid (Abb. 7a bis c). Die Struktur der herangewachsenen Kerne ist, solange der

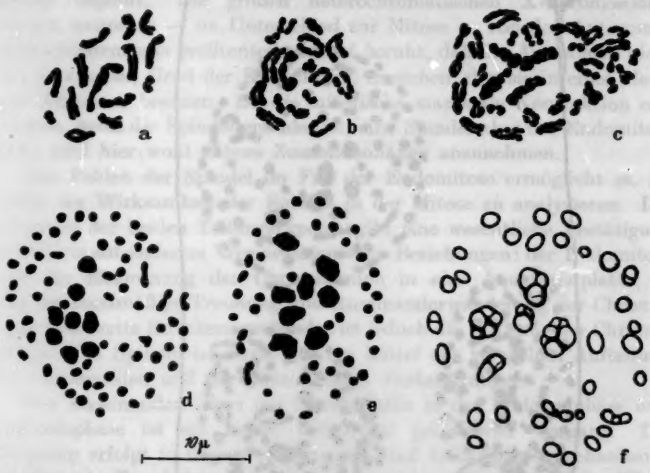

Abb. 7a—f. Verschiedene Entwicklungsstadien der Kerne der Mitteldarmepithelzellen, V. Stadium und Imago. e aus Weibchen, die anderen aus Männchen. a Endoanaphase eines diploiden Kerns; b Endotelophase eines diploiden Kerns; c Endotelophase eines oktoploiden Kerns; d, e fertiggestellte Kerne (im Weibchen je 2 X-Chromosomen vereinigt); f Stadium der Chromosomenvakuolisierung.

Stäbchensaum noch erhalten ist, mannigfachen Veränderungen unterworfen. Besonders auffallend ist ein Stadium stärkster Vakuolisierung aller Chromosomen (Abb. 7f) und ein wahrscheinlich vorangehendes Stadium schwächster chromatischer Färbbarkeit. Beide Ausbildungsweisen finden sich in keiner anderen Kernart, und sie stehen zweifellos in bestimmter Beziehung zur Funktion.

IV. Allgemeines.

Die Untersuchungen zeigen, daß die ursprünglich (1937) gegebene Deutung des Zustandekommens der Polyploidie auf dem Wege von Kernverschmelzungen oder durch Restitutionskernbildung (Vereinigung der Tochterplatten einer gewöhnlichen Mitose in einem Kern) nicht aufrechterhalten werden kann. In der zweiten Mitteilung (1938a) wurde

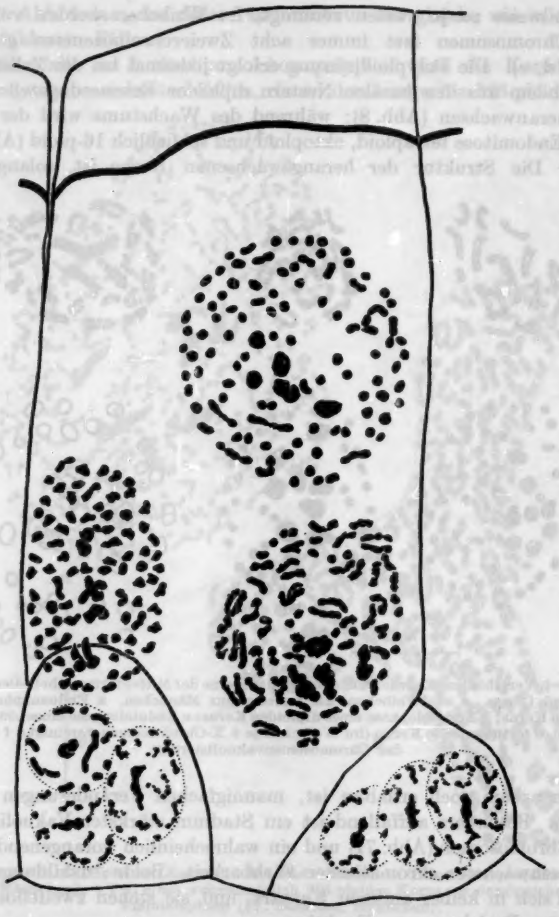

Abb. 8. Teil eines Querschnitts durch das Mitteldarmepithel, oben der Stäbchensaum unten die Basis; Zellgrenzen nur teilweise angedeutet; V. Stadium, Männchen. An der Basis 2 Nester von Erneuerungszellen mit diploiden Kernen (links oben einer in Prophase). Rechts Mitte letzte Endotelophase (4 gespaltene X-Chromosomen und 160, größtenteils noch einander genäherte oder zusammenhängende, nicht rekonstruierte Autosomen); links ein oktoploider Kern bald nach der Endotelophase, rechts oben ein fertiggestellter Kern (2 einzelne X-Chromosomen, die anderen 6 zu je zweien vereinigt).

die Unrichtigkeit der Annahme daraus erschlossen, daß ein wiederholter Verschmelzungsvorgang und überhaupt eine Spindelbildung im

Fall sehr hochpolyploider Kerne, im besonderen der Speicheldrüsenkerne, unvorstellbar ist.

An die Stelle von Vermutungen tritt nunmehr die klare Vorstellung der Endomitose. Der Ablauf kann in den Grundzügen als geklärt gelten; für eine letzte Auswertung sind die mit Alkohol-Eisessig und Essigkarmin behandelten Präparate allerdings nicht geeignet.

Bezeichnend für die Endomitose ist, daß sie wie eine gewöhnliche Mitose beginnt. Die großen heterochromatischen X-Chromosomen bleiben weiterhin — im Unterschied zur Mitose — von den Autosomen unterschieden, was größtenteils darauf beruht, daß die Autosomen nicht den maximalen Grad der Kontraktion erreichen, den sie in einer Meta- und Anaphase besitzen. Da die mitotische maximale Kontraktion erst auftritt, wenn die Spindel gebildet ist, eine Spindel aber der Endomitose fehlt, sind hier wohl nähere Zusammenhänge anzunehmen.

Das Fehlen der Spindel im Fall der Endomitose ermöglicht es, indirekt die Wirksamkeit der Spindel in der Mitose zu analysieren. Der Vergleich der beiden Teilungstypen ergibt eine wesentliche Bestätigung der bisher auf anderem Weg erschlossenen Beziehungen: der Endomitose *fehlt* die Einordnung der Chromosomen in eine Äquatorialplatte, es *fehlt* die regelmäßige Trennung und Auseinanderwanderung der Chromatiden über weite Strecken; *vorhanden* ist jedoch die Spaltung der Chromosomen. Die Spindel ist somit nur ein Mittel der geregelten Aufteilung der Chromatiden und der dazu nötigen Vorbereitungen.

Das Auseinanderrücken der Chromatiden in der Endoanaphase und Endotelophase ist ein bisher unbekannt gebliebener Vorgang. Die Trennung erfolgt in dem Sinn autonom, daß kein Spindelmechanismus beteiligt ist. Es scheint, daß das Sich-Trennen auch nicht auf der Wirksamkeit des Spindelansatzes (Centromers) beruht, da die sich trennenden Chromatiden am längsten an den Enden aneinander haften bleiben und andererseits zufolge der Analyse der meiotischen Anaphasen terminale Insertion vorhanden ist (1937) [1]. Der Spindelansatz erscheint vielmehr inaktiv. Dies, wie die Unterdrückung der Spindelbildung sind die wesentlichen Merkmale der Mitosen, die sich bei Pflanzen durch Behandlung mit Colchicin auslösen lassen (vgl. z. B. Levan 1938). Auch das Endergebnis — Polyploidie — ist das gleiche. Ein Unterschied besteht jedoch darin, daß bei der Colchicinmitose normale metaphasische Kontraktion erfolgt, im Fall der Endomitose aber nicht.

Bezeichnend ist, daß der Übergang zur Telophase ganz allmählich erfolgt: die Chromatidentrennung dauert noch an, wenn bereits die Umbildung zur Ruhekernstruktur eingesetzt hat, und die endweise Verbindung kann an einzelnen Chromosomen sogar bis zur völligen

[1] Eine Analyse der somatischen Anaphasen ist in dieser Hinsicht ausgeschlossen, da das Auseinanderwandern unter „Parallelverschiebung", d. h. ohne Nachziehen der Arme geschieht (vgl. 1937).

Kernruhe erhalten bleiben; es läßt sich demnach gar keine scharfe Grenze zwischen Endoana- und Endotelophase ziehen. Die X-Chromosomen nehmen eine Ausnahmestellung insofern ein, als sich ihre Chromatiden später als die der Autosomen, oft erst in der nächsten Teilung, voneinander lösen; die Ursache liegt in ihrer heterochromatischen Beschaffenheit.

Auch nach der Auseinanderlösung bleiben die Chromatiden noch nebeneinander im Kern liegen. Dies gilt nicht nur für die X-Chromosomen, an welchen sich die Erscheinung durch zahlreiche Teilungen hindurch leicht beobachten läßt, sondern auch für die Autosomen; so findet man bei aufmerksamer Betrachtung der Endotelophasen in polyploiden Kernen leicht Stellen, an welchen zwei oder auch mehr Chromatidenpaare gleicher Größe nebeneinander liegen; sie sind offenbar in früheren Endomitosen auseinander entstanden. Daß aber doch gewisse Bewegungen im Kernsaft stattfinden und die Chromosomen mit sich führen, ergibt sich daraus, daß andernfalls in hochpolyploiden Kernen Areale von Chromosomenabkömmlingen bestimmter Größe auftreten müßten, was nicht der Fall ist; auch könnten in hochpolyploiden Kernen nicht mehr als höchstens zwei Nukleolen vorhanden sein, was auch nicht zutrifft[1].

Daß die Endomitose das einzige Mittel der Polyploidisierung ist, ist sehr wahrscheinlich, läßt sich aber zur Zeit nicht beweisen. Sicher ist sie aber der *gewöhnliche* Vorgang, der die Polyploidie verursacht. Daß andererseits polyploide Kerne in bestimmten Geweben vielleicht auch durch Restitution entstehen können, läßt sich auf Grund des Auftretens unregelmäßiger polyploider Mitosen für möglich halten (Abb. 9d bis g). Während tetraploide und manchmal auch oktoploide Mitosen normal wie diploide Mitosen ablaufen können (Abb. 9a—c), sind manche oktoploide und alle höher polyploiden Kerne anscheinend nicht mehr fähig, in der Metaphase normale zweipolige Spindeln auszubilden: es entstehen vielmehr mehrpolige Spindeln, in denen die Äquatorialplatten entsprechend gebogen und vielfach auch ziemlich unregelmäßig gestaltet sind (Abb. 9d—g). Solche Teilungsfiguren findet man im Fettkörper und in den als Oenocyten bezeichneten Zellen. Die höchste Chromosomenzahl beträgt schätzungsweise 320, was einem 32-ploiden Kern entspricht (eine genaue Zählung ist infolge der Verbiegung der Platten ausgeschlossen).

Was aus diesen Teilungsfiguren wird, konnte bisher allerdings nicht festgestellt werden, da keine späteren als Metaphasestadien beobachtet

[1] Zur vollständigen Beschreibung der Endomitose würde noch das Verhalten der Centrosomen gehören. Das Auftreten mehrpoliger Mitosespindeln in niedriger polyploiden Kernen (Abb. 9e) deutet an, daß wenigstens in diesen Fällen eine Vermehrung der Centrosomen erfolgt ist. Einen näheren Einblick zu gewinnen ist mittels der Essigkarmintechnik nicht möglich.

wurden. Daß die Spindel sekundär zweipolig werden könnte, scheint fast ausgeschlossen; es besteht also die Möglichkeit, daß eine Aufteilung auf mehr als zwei Tochterkerne eintritt oder daß *Restitutionsbildung* erfolgt. Um den normalen Weg der Polyploidisierung kann es sich dabei aber jedenfalls nicht handeln; denn 1. sind die Endomitosen ungleich häufiger, und 2. kommen höher als 32-ploide Mitosen nicht vor. Schon

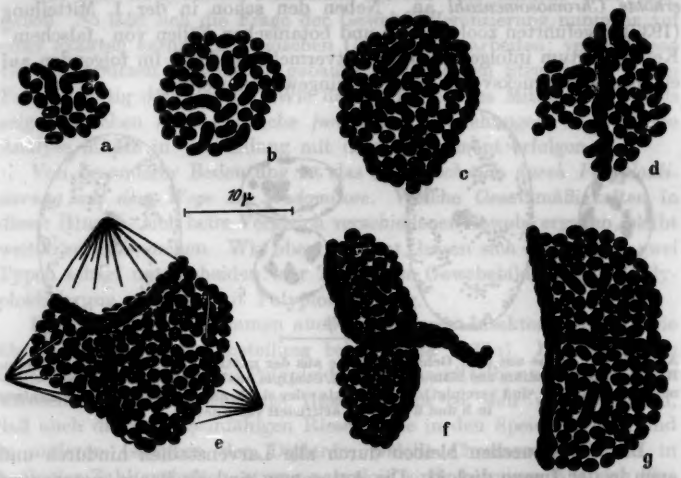

Abb. 9a—g. Metaphaseplatten im Fettkörper und in Oenocyten (?). a diploid, ♀; b tetraploid, ♀; c oktoploid, ♀ (rechter Rand etwas aufgekrümmt); d oktoploid, ♂ (Platte stark verbogen); e, f, g 16- oder 32-ploid, ♂, Spindel mehrpolig (nur in e dargestellt).

diese machen infolge der Verbiegungen der Platten einen pathologischen Eindruck, und es ist anzunehmen, daß diese Teilungsfiguren mit ihrem Ballast von Chromosomen das äußerste sind, was sich mit dem Mitosemechanismus eben noch verträgt. Die Polyploidisierung auf dem Weg der Endomitosen geht aber viel weiter.

Das Auftreten polyploider Mitosen zeigt jedenfalls, sofern noch irgendein Zweifel bestehen könnte, daß die betreffenden Kerne tatsächlich polyploid sind. Für die nicht mitotisch teilungsfähigen Kerne ergibt sich die Polyploidie, wie früher geschildert, zwingend aus den unmittelbar zu beobachtenden Strukturen. In dieser Hinsicht sind die Kerne der Wanzen, im besonderen die von *Gerris lateralis*, allen anderen Objekten, für welche Polyploidie behauptet wurde (Dipteren, vgl. Berger und Bauer), weit überlegen. Der methodische Vorteil gegenüber Fällen,

wo nur aus der Messung der Kerngröße Schlüsse auf „innere Teilungen" gezogen werden können (JACOBJ u. a.), liegt auf der Hand.

Mit der Erhöhung der Chromosomenzahl erfolgt auch bei *Gerris* eine Vergrößerung des Kernvolumens (eine exakte Messung ist übrigens fast immer infolge der unregelmäßigen Kernform ausgeschlossen). *Umgekehrt zeigt aber ein größeres Kernvolumen nicht notwendigerweise eine erhöhte Chromosomenzahl* an. Neben den schon in der 1. Mitteilung (1937) angeführten zoologischen und botanischen Fällen von „falschem" Kernwachstum infolge von Kernsaftvermehrung kann im folgenden auf ein neues eindrucksvolles Beispiel hingewiesen werden.

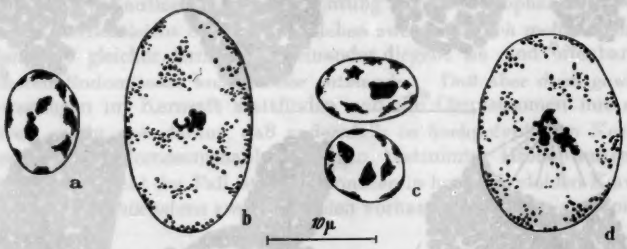

Abb. 10a—d. Kerne aus dem Gehirn. a und c aus der mittleren, b und d aus der äußeren Region; a, b V. Stadium aus Männchen, c, d II. Stadium aus Weibchen (die beiden X-Chromosomen in c oben sind vereinigt). Die Struktur der stark aufgelockerten Chromozentren in b und d ist wohl artifiziell verändert.

Die Ganglienzellen bleiben durch alle Larvenstadien hindurch und auch in der Imago diploid. Die Autosomen sind als flache, vorwiegend der Kernwand angelagerte Chromozentren ausgebildet, das X-Chromosom oder die beiden X-Chromosomen liegen zentral. Im besonderen im Gehirn kommen neben Kernen normaler Größe auch stark vergrößerte Kerne vor (letztere in den peripheren Teilen). Diese Vergrößerung kommt ausschließlich durch *Kernsaftvermehrung* zustande (Abb. 10). Sie hat neben der „Aufblähung" des gesamten Kerns zur Folge, daß die Chromosomen aufgelockerter, gewissermaßen „zerstäubt" werden. Diese Veränderung geht bei den Autosomen viel weiter als bei den heterochromatischen X-Chromosomen; die Autosomen werden, offenbar infolge starker Quellung, im äußersten Fall fast unsichtbar bzw. erscheinen aus kleinen, chromomerenartigen Körnchen aufgebaut.

An Stelle von Endomitosen sind im Gehirn in allen Stadien zahlreiche Mitosen zu sehen. Das Massenwachstum erfolgt also in diesem Fall durch Vermehrung der embryonalen, diploiden Zellen. Das Wachstum anderer Gewebe, in extremem Maß z. B. der Speicheldrüse, geschieht dagegen ohne Zellteilung durch Vergrößerung der Zellvolumina unter Polyploidisierung der Kerne. Es handelt sich also um zwei grundsätzlich verschiedene Bildungsweisen und Bauprinzipien. Inwieweit in

anderen Fällen auch Mischtypen vorkommen, bliebe zu untersuchen. Einen Sonderfall stellt das Epithel des Mitteldarms mit seinen sich mitotisch vermehrenden Erneuerungszellen und den im Lauf einer Funktionsperiode 16-ploid werdenden Drüsenzellen dar.

V. Ausblicke.

Aus den mitgeteilten Tatsachen ergeben sich manche neue Aufgaben. So läßt sich die Frage der Gewebedifferenzierung nunmehr auf einer exakten kernmorphologischen Grundlage bearbeiten: verschiedene Gewebe besitzen verschieden gebaute Kerne, deren Struktur in jedem Fall eindeutig definiert ist. Wie das Verhalten des Mitteldarmepithels zeigt, bestehen auch deutliche *funktionelle* Beziehungen; ihre nähere Analyse müßte in Verbindung mit dem Experiment erfolgen.

Von besonderer Bedeutung ist das *Kernwachstum durch Polyploidisierung auf dem Wege der Endomitose*. Welche Gesetzmäßigkeiten in dieser Hinsicht sich beim Vergleich verschiedener Gewebe ergeben, bleibt weiter zu untersuchen. Wie oben erwähnt, lassen sich schon jetzt zwei Typen scharf unterscheiden: der Typus der Gewebebildung ohne Polyploidisierung und der mit Polyploidisierung.

Polyploide Kerne kommen auch bei anderen Insekten vor (vgl. die übersichtliche Zusammenstellung bei Berger 1938a). Im besonderen bei Dipteren läßt sich die Polyploidie dann unmittelbar erkennen, wenn Mitosen eintreten (neuerdings Berger). Es ist sehr wahrscheinlich, daß auch die teilungsunfähigen Riesenkerne in den Speicheldrüsen und in anderen Geweben der Dipteren, welche Chromosomenbündel in haploider Zahl enthalten, streng polyploid sind; beweisen läßt sich dies allerdings deshalb nicht, weil die Anzahl der Einzelelemente in einem Bündel nicht unmittelbar festgestellt werden kann und es daher fraglich bleibt, ob die Zahlen wirklich Potenzen von zwei sind; als sicher kann es aber gelten, daß eine Vermehrung der Chromosomen (Chromonemata) während des Heranwachsens dieser Kerne erfolgt, daß das Kernwachstum also auf einer wiederholten Teilung der Chromosomen im „Ruhekern" beruht. Wieweit diese Art des Kernwachstums bei anderen Organismen, auch bei Pflanzen, verbreitet ist, läßt sich noch nicht absehen. Wenn man auch geneigt ist, anzunehmen, daß sie *jedem* echten, d. h. mit Chromatinvermehrung verbundenen Kernwachstum zugrunde liegt, so fehlen hierfür doch noch die nötigen Anhaltspunkte. Für die Aracee *Sauromatum guttatum*, die in bestimmten Organen ein beträchtliches echtes Kernwachstum besitzt, läßt sich eher das Gegenteil annehmen (Geitler 1938c; daselbst auch weitere botanische Angaben).

Welche Beziehungen zwischen Polyploidisierung und dem allein durch Messungen (hauptsächlich an Säugern) festgestellten „rhythmischen Kernwachstum" (Jacobj u. a.) bestehen, müßte erst klargelegt werden. Im Hinblick auf die sehr verschiedenen gefundenen Zahlenreihen,

die oft stark von der Reihe 1:2:4:8 usw. abweichen, wäre an einem Objekt, das den Polyploidiegrad der Kerne sicher erkennen läßt und gleichzeitig sichere Messungen des Volumens zuläßt, zu erforschen, wie die Chromosomenvermehrung auf die Volumzunahme wirkt.

In diesem Zusammenhang wäre auch die Frage zu erörtern, ob es mehrwertige Chromosomen, wie sie G. Hertwig postuliert hat, gibt. Bei *Gerris* (und anderen Wanzen) fehlt jeder Anhaltspunkt für eine derartige Erscheinung: die Chromosomen sind in verschiedenen Mitosen

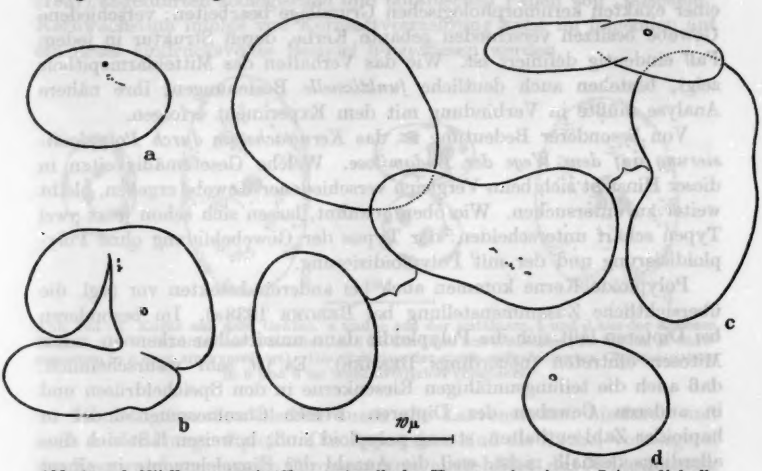

Abb. 11a—d. *Nitella mucronata* (Characee). a flacher Kern aus einer jungen Internodialzelle, oben das Chromozentrum, darunter eine wolkige heterochromatische Struktur; b Kern aus einer älteren Internodialzelle in Zerfall (Chromozentrum vakuolisiert); c durch Zerfall entstandene 5 Kernstücke aus einer noch älteren Internodialzelle (der obere rechts in Kantenansicht mit dem Chromozentrum); d eines von 18 Kernfragmenten aus einer ganz alten Internodialzelle; es enthält das Chromozentrum. — Alk.-Eisessig, Essigkarmin.

nur wenig verschieden groß, und vor allem fehlt die postulierte Aufspaltung in Einzelelemente im Laufe mehrerer schnell aufeinanderfolgender Teilungen ohne Wachstum. Die bei den Dipteren vorhandenen Chromosomenbündel lassen sich, wie auch Berger (1938a) betont, nicht in diesem Sinn deuten; sie sind vielmehr ein Sonderfall, der durch die den Dipteren eigentümliche somatische Paarung verursacht ist (vgl. für die weitere Deutung Geitler 1938a). Hieraus erklärt sich auch die Möglichkeit der von Berger im Enddarm während der Verpuppung beobachteten Erscheinung der „somatischen Reduktion", bei der eine Zerlegung der Bündel in die Einzelchromosomen während aufeinanderfolgender Mitosen stattfindet[1].

[1] Das Verhalten steht offenbar in Beziehung zu der Art der Metamorphose (Verpuppung unter weitgehender Veränderung der Gewebe). Bei den Wanzen fehlen derartige Erscheinungen vollkommen.

Der Nachweis von Endomitosen läßt schließlich eine erneute Analyse der Strukturänderungen in *amitotisch* sich teilenden Kernen wünschenswert erscheinen. Obwohl im Fall der Endomitose der Wanzen eine bestimmte Orientierung der Tochterchromosomen in bezug auf den gesamten Kern nicht vorhanden ist, scheint es doch nicht ausgeschlossen, daß in anderen Fällen eine solche eintritt und in Verbindung mit einer Kerndurchschnürung eine erbgleiche Teilung ermöglicht. Eine solche Untersuchung wäre aussichtsreich in jenen Fällen, wo die Amitose einen gesetzmäßigen Vorgang darstellt, so vor allem bei den Ciliaten, aber auch in bestimmten Metazoengeweben (vgl. zum 1. Fall POLJANSKY, zu dem letzteren Fall FISCHER und RIES). In anderen Fällen handelt es sich dagegen sicher nicht um Amitosen im eigentlichen Sinn, sondern um *Kernzerfall*. Hierfür geben die Characeen gute Beispiele ab; so läßt sich für *Nitella mucronata* nachweisen, daß das einzige Chromozentrum des Ausgangskerns in jungen Internodialzellen einem, und nur einem, der Tochterkernstücke in den alten Zellen zugeteilt wird (Abb. 11). Diese zerfallenden Kerne besitzen auch kein oder nur geringfügiges Wachstum durch Chromatinvermehrung.

Zusammenfassung.

Das Erhaltenbleiben distinkter Chromosomen bzw. Chromozentren im Ruhekern und das gesetzmäßige Vorkommen von polyploiden Kernen in bestimmten Geweben der Heteropteren ließ sich an 21 Arten aus 17 Gattungen und 8 Familien feststellen.

Die Polyploidie entsteht regelmäßig (und wahrscheinlich ausschließlich) durch eine Art von Mitose im Innern des Kerns (Endomitose). Während dieses Vorgangs durchlaufen die Chromosomen Veränderungen, die denen einer gewöhnlichen Mitose ähneln (Endoprophase, Endometaphase usw.); doch fehlt das Stadium der stärksten metaphasischen Kontraktion.

Der wesentliche Unterschied gegenüber der Mitose besteht in dem Ausbleiben der Spindelbildung. Dies hat zur Folge, daß keine gerichteten Bewegungen der Chromatidenpaare und der Chromatiden eintreten und daß keine Kernteilung durchgeführt wird. Die Trennung der Schwesterchromatiden erfolgt in einem Kern synchron, jedoch im Vergleich zu einer gewöhnlichen Anaphase regellos; bezeichnend ist das langdauernde Haftenbleiben der Schwesterchromatiden an den Enden. Die Spindelansatzstelle ist offensichtlich inaktiv. Es besteht im ganzen eine bemerkenswerte Übereinstimmung mit der Colchicinmitose.

Der Vorgang der Endomitose zeigt unmittelbar, daß eine vollständige Chromosomenteilung ohne Spindelapparat durchgeführt werden kann. Der für Dipterenkerne aus dem Endergebnis erschlossene Vorgang des Kernwachstums durch Polyploidisierung läßt sich hier unmittelbar und schrittweise verfolgen.

Endomitosen treten in ganz verschiedenen Organen und Geweben auf, nämlich in den MALPIGHIschen Gefäßen, den Hodensepten, dem Follikelepithel, dem Fettkörper, den als Oenocyten bezeichneten Zellen, der Speicheldrüse, Bindegewebe und im Epithel des Mitteldarms. Im letzteren Fall erfolgt die Polyploidisierung vor jeder Funktionsperiode der Drüsenzellen während des Heranwachsens der diploiden Erneuerungszellen.

Im Unterschied zu Geweben, deren Massenzunahme auf Zellwachstum unter endomitotischer Polyploidisierung der Kerne beruht, gibt es auch Gewebe, deren Massenzunahme durch Teilung diploid bleibender Zellen zustande kommt (Epidermis, Ganglien). Die beträchtliche Größenzunahme der diploiden Kerne bestimmter Zonen im Gehirn wird ausschließlich durch Kernsaftvermehrung verursacht.

Aus diesen Tatsachen ergeben sich Ausblicke für die Bearbeitung der Probleme Kernbau und Gewebedifferenzierung, Kernbau und Funktion, Endomitose und Kernwachstum, Endomidose und Amitose.

Literatur.

Bauer, H.: Cytologie. Fortschr. Zool. **1/2** (1937). — Die polyploide Natur der Riesenchromosomen. Naturwiss. **26** (1938). — **Berger, Ch. A.:** Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito, *Culex pipiens*. Publ.-Nr 496, Carnegie Inst. Wash., Contrib. to Embryol. **1938a**, Nr 167. — Prophase chromosome behavior in the division of cells with multiple chromosome complexes. J. Hered. **29** (1938b). — **Fischer, Ilse:** Über den Wachstumsrhythmus des Follikelepithels der Läuse und Federlinge und seine Beziehungen zum Arbeitsrhythmus der Zellen und zur Amitose. Z. Zellforsch. **23** (1935). — **Geitler, L.:** Die Analyse des Kernbaus und der Kernteilung der Wasserläufer *Gerris lateralis* und *Gerris lacustris* und die Somadifferenzierung. Z. Zellforsch. **56** (1937). — Über den Bau des Ruhekerns mit besonderer Berücksichtigung der Heteropteren und Dipteren. Biol. Zbl. **58** (1938a). — Die Entstehung der polyploiden somatischen Zellkerne bei Heteropteren durch wiederholte Chromosomenteilung ohne Spindelbildung und Kernteilung. Naturwiss. **26** (1938b). — Über das Wachstum von Chromozentrenkernen und zweierlei Heterochromatin bei Blütenpflanzen. Z. Zellforsch. **28** (1938c). — **Heitz, E.:** Die Herkunft der Chromozentren. Planta (Berl.) **18** (1932). — **Hertwig, G.:** Die Vielwertigkeit der Speicheldrüsenkerne und Chromosomen bei *Drosophila melanogaster*. Z. Abstammungslehre **70** (1935). — **Jacobj, W.:** Die Zellkerngröße beim Menschen. Z. mikrosk.-anat. Forsch. **38** (1935). — **Levan, A.:** The effect of colchicine on root mitoses in *Allium*. Hereditas **24** (1938). — **Mitis, H. v.:** Ökologie und Larvenentwicklung der mitteleuropäischen *Gerris*-Arten. Zool. Jb., System. **69** (1937). — **Poljansky, G.:** Geschlechtsprozesse bei *Bursaria truncatella*. Arch. Protistenkde **81** (1933). — **Ries, E.:** Entwicklungs- und Differenzierungsperioden im Leben der Zelle. Naturwiss. **25** (1937).

(John Innes Horticultural Institution Merton.)

THE MEASUREMENT OF PACKING AND CONTRACTION IN CHROMOSOMES.

By

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With 2 figures in the text.

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The internal changes that chromosomes undergo in the course of mitosis and meiosis have now been shown to follow a common type in a wide range of Plants, Animals and Protista. Briefly the nuclear cycle may be said to be a spiralisation cycle. This cycle must depend on molecular properties of the chromosomes, properties which are universal, and which must therefore be understood before a molecular model of the chromosome can be put forward.

One of the most obvious tests to apply is to measure the changes in length and internal arrangement of chromosomes during mitosis and also in the indefinite or unlimited resting stages and prophases that are found in special tissues. We can then see what assumptions of geometrical or stereochemical relationships these measurements agree with. We need quantitative data whether exact or approximate. Many workers have recently made observations that serve this purpose. Some of their records are in the form of measurements, others in the form of photographs and drawings from which measurements can be made. We are here attempting to abstract some of the results, as a foundation for comparative study.

Before considering these measurements let us recall the broad conditions of observation. There are five well-known, more or less stable and accurately measurable states of the chromosome in higher organisms. These are, in order of increasing extension:

(I) first metaphase of meiosis,

(II) metaphase of mitosis,

(III) the pachytene stage of the prophase of meiosis,

(IV) the highly extended or diffuse state found at diplotene in the oögenesis of vertebrates,

(V) the resting nuclei found particularly in salivary gland cells of Diptera.

The greatest range of extension is found between diakinesis or first metaphase of meiosis (cf. Darlington, 1934) and the salivary gland chromosomes in Drosophila, where taking Bridges' measurements (1935) a range of 300:1 may be assumed. A similar degree of extension

Table 1. Measurements of Chromosome lengths during Spiralisation (in microns).

No.	Organism	Chromosomes	Pachytene	Diplotene	Diakinesis	M-A I	M-A II	Mitosis A (P.G.)	Mitosis B (R.T.)	Sp. Coeff. (Meiosis)	Author
	Animals:										
1	*Actinophrys* (Heliozoa)	LII	c. 30	c. 7.5	—	1.0	1.5	—	—	30.0	Belar, 1926
2	*Aggregata* (Coccidia)	LII	50	—	—	6.5	—	20.0	1.5	7.7	Belar, 1926
3	*Pristiurus* (Pisces)	♀ LII	—	c. 100	14	2.0	—	—	—	50.0	Rückert, 1892
4	*Gallus* (Aves)	♀ 3LII	—	c. 100	—	11.5	—	—	15	8.7	White, 1932 and unpub. cf. Koltzoff, 1938
5	*Dasyurus* (Marsupialia)	♂♂ 6II	72	80	—	—	20	—	29	4.0	Koller, 1936
6	*Sarcophilus* (Marsupialia)	6II	79	69	—	—	19	—	27	4.2	Koller, 1936
7	*Chorthippus* (Orthoptera)	8II	290	78	50	30	29	49	35	10.0	Darlington, 1936
7a	*Chorthippus* (Orthoptera)	♂ X	6	6	5	3	—	6.5	4.2	—	Darlington, 1936
8	*Aranea* (Araneida)	♂♂ 11II	149	34	21	19	18	—	—	8.3	Pätau, unpub.
9	*Aranea* (Araneida)	♀ 11II	133	—	—	14.6	—	24	13	9.1	Pätau, unpub.
	Plants:										
10	*Tulipa Hageri*	12II	792	149	102	42	50	85	c. 98	18.8	Upcott, 1939 and unpub.
11	*T. silvestris*	24II	1512	—	—	109	136	190	—	13.9	Upcott, 1939
12	*Kniphofia aloides*	5II	c. 400	111	50	41	—	46	—	9.9	Darlington, 1933
13	*Bellevalia romana*	4II	287	—	—	32	—	—	44	8.8	Dark, 1934
14	*Trillium Kamtschaticum*	5II	593	—	—	61	—	—	—	9.7	Matsuura, 1934
15	*Lilium* spp.	12II	1469	257	152	159	—	—	c. 190	9.7	Belling, 1928
16	*L. regale*	12II	996	—	—	144	—	180	264	6.9	Sax and Sax, 1935
17	*Tradescantia paludosa*	6II	486	—	—	54	—	72	126	9.0	Sax and Sax, 1935
18	*Vicia Faba*	6II	588	—	—	54	—	66	78	10.9	Sax and Sax, 1935
19	*Secale cereale*	7II	427	—	—	56	—	—	98	7.6	Sax and Sax, 1935
20	*Zea Mays*	No. 9	60	—	5	4.5	—	2.8	2.6	13.3	McClintock, 1933
21	*Zea Mays*	BL	30	—	3	3.3	—	2.2	1.8	10.0	Darlington, 1937a
22	*Zea Mays*	BS	—	—	—	2.0	—	1.5	1.2	—	Upcott, unpub.

Notes on Table I.

General. The pachytene measurements, when taken from projections of the chromosomes lying in spherical unpressed nuclei, are multiplied by a factor $4/\pi$ to give an average correction for the whole nucleus (PĀTAU, 1937). This does not apply to 15—21 where chromosomes have been flattened in preparation.

Mitotic measurements are for metaphase. They are given for early and late spermatogonial or oogonial stages in animals (A and B) and for pollen grains (PG) and root tips (RT) in plants.

At metaphase of meiosis (MI) the tension of the separating centromeres and the presence of loops between successive chiasmata makes the measurement somewhat inaccurate. Wherever possible therefore the measurements have been made at first anaphase (AI), or in the case of *Zea mays* on univalents at metaphase. Preparations of anaphase were not available for *Aranea*, *Kniphofia* or *Bellevalia*, so measurements made at metaphase have been included instead in the table.

The Spiralisation Coefficient (Sp. Coeff.) given as a basis for comparison is the factor of linear contraction from the longest to the shortest stage of meiosis.

Special. 1. First figure is an estimate for leptotene. L II refers to the longest bivalent.

2. Mitosis (A) has chromosomes uncontracted in very rapid divisions. (B) has chromosomes much shorter than at meiosis.

3 and 4. "Lamp-brush" chromosomes in the indefinitely prolonged diplotene stage. 4: the diplotene figures are taken from nuclei at a third their maximum volume, and according to Mr. WHITE the chromosomes grow later to a much greater length. The first metaphase length is taken from the spermatocytes.

5. The diplotene chromosomes are extending as they pass into a diffuse stage, and are therefore longer than at pachytene. The spiralisation coefficient is taken from the second metaphase chromosomes which are the minimum length. It is the smallest known in any organism.

7. Chromosomes contract during pachytene. X chromosome remains in approximate metaphase condition throughout resting stage as in the Flagellata.

9 and 10. Measurements have been made on preparations fixed in chromosmic solution and stained in gentian violet.

15. The chromosomes increase in length during pachytene.

20—22. Root-tip chromosomes contracted by fixation in chrom-osmic. Meiotic chromosomes swollen by pre-fixation in acetic alcohol. The three series of measurements are therefore not comparable.

21, 22. The inert B chromossomes show a spiralisation comparable to that of the active chromosomes at both mitosis and meiosis. The dissimilar extension of active and inert parts of chromosomes in the salivary glands cells of Diptera therefore seems to be peculiar to these cells.

may be secured by manipulation: the chromosomes of the frog can be stretched to 500 micra (DURYEE, 1937). Almost all the stages that lead up to this maximum stretching can be made out from particular stages in different organisms. The diffuse diplotene nuclei show an extension of 50:1 or, as we may say, a spiralisation coefficient for meiosis of 50. Pachytene in *Tulipa* shows 19:1 and in *Lilium* 7:1.

The values for early prophase of mitosis are more difficult to obtain. All the measurements given so far indicate a less length than at pachytene. The earliest decipherable stage is more contracted as well as more difficult to measure than pachytene, for a reason that we shall consider

later. We are therefore safer in taking the pachytene lengths as our standard. We can see that at metaphase of mitosis a contraction lower than 4:1 is found in *Lilium regale*, while elsewhere a complete range up to 20:1 is found. Taking the early prophase of mitosis as a standard Sax and Sax (1935) found contractions in mitosis as low as 1:2.5.

When we turn to the Protozoa we find a corresponding type of mitosis in certain species. Pätau (1937) recorded a contraction of 1:5.5 in

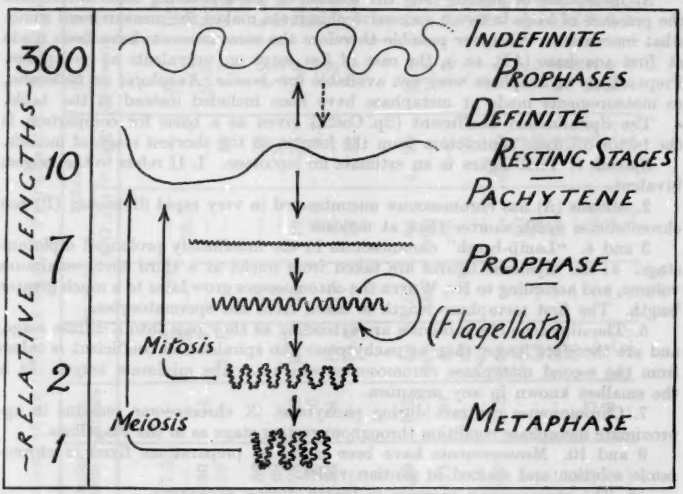

Fig. 1. Diagram to represent the possible spiralisation relationships of chromosomes with different degrees of elongation: (I) in salivary glands or diffuse diplotene stages, (II) in resting stages or at pachytene, (III) and (IV) at prophase, (V) at mitotic metaphase, (VI) at meiotic metaphase. The necessary assumptions are an inter-chromomere spiral at pachytene and a *continuous* development of large-scale spirals from smaller scale spirals as opposed to the *discontinuous* development of major spirals superimposed on minor spirals.

Merodinium between prophase and telophase. On the other hand we also find that the whole range of behaviour in the higher organisms can be shown in the cycle of one species. In *Aggregata Eberthi* (cf. Belar 1926) the contraction at mitosis seems to vary from 2.5:1 to 33:1. In certain Flagellata a still more remarkable situation arises, where the chromosomes retain a constant degree of spiralisation throughout the mitotic cycle (Cleveland 1938). The distinction that has been drawn between an internal spiral and a relic spiral then breaks down.

Associated with these changes of length, and in part at least, responsible for them is the coiling and uncoiling of the chromosome thread to do and undo an optically homogeneous spiral. This internal spiral exists in two orders of size that are visible to us at first metaphase of

meiosis — the major and minor spirals, which can be successively unravelled by heat or by KUWADA's chemical treatment.

In order to decide what part the formation of these spirals plays in the shortening of the chromosomes, two methods of analysis have been successfully applied. The first is simply morphological. It is best to begin with the mitotic chromatid at metaphase or anaphase where a single thread is apparently coiled in a single spiral. It will be seen that the measurements of the number of coils, *n*, of diameter, *d*, in relation to the diameter (D) of the chromatids measured (or illustrated) by several workers agree in showing in value for what we may call the packing factor D/d of 2.0 to 2.5 (Table 2). This value represents an

Table 2. Calculation of Packing Factor D/d.

No.	Author	Method	Stage	Material	L	n	d	D	D/d
1	GEITLER 1935 (Fig. 2e)	aceto-carmine	pollen-grain M.	*Gasteria*	18	20	0.9	1.8	2.0
2	UPCOTT 1935	aceto-carmine	pollen tube M.	*Tulipa*	17	33	0.52	1.2	2.3
3	DARLINGTON and LA COUR 1938	FLEMMING, non-acetic	root tip M.	*Paris "D"*	22	37	0.59	1.2	2.0
4	GEITLER 1938 (Fig. 1a)	acetic acid	nucellus M.	*Paris*	12	26	0.46	1.0	2.2
5	PÄTAU 1939	aceto-carmine	anther wall A.	*Tradescantia* (2x)	19	30	0.63	1.4	2.1
6	PÄTAU 1939	aceto-carmine	pollen grain A.	*Tradescantia* (2x)	15	25	0.59	1.5	2.5
7	PÄTAU 1939	aceto-carmine	pollen grain A.	*Tradescantia* (4x—2)	13	22	0.62	1.3	2.1
8	PÄTAU 1939	aceto-carmine	pollen mother cell (AII)	*Tradescantia* (4x—2)	11	21	0.54	1.4	2.5
9	SAX[1]	aceto-carmine	pollen mother cell (AII)	*Tradescantia* (2x)	8	11	0.75	1.4	1.9
10	SAX and HUMPHREY	alcohol + acetic carmine	pollen mother cell (MII)	*Tradescantia* (2x)	13	13	1.0	1.8	1.8

M, Metaphase; A, anaphase; for L, n, d, and D cf. Fig. 2.

[1] Figure in STRAUB, 1938; magnification of 2000 assumed.

arrangement such as that shown in the top and bottom middle diagrams of Fig. 2.

Will the coiling of a thread into such a spiral without any change in its internal dimensions alone account for the shortening observed? Clearly any simple spatial rearrangement which doubles the diameter of the chromatid while the chromatid remains solid, will account for a reduction of length to about one quarter. Since the thread seems to have reached more than double its diameter in coiling ($D/d > 2$) a greater reduction than to a quarter is accounted for and in this simple and approximate sense we may say that the spirals observed in *Tulipa*, *Paris*, *Gasteria* and *Tradescantia* agree with the recorded contractions in length.

At meiosis, where a major spiral and minor spiral occur together, the situation is also complicated by the joint coiling of the two chromatids at metaphase. At anaphase however similar conditions to those

found at mitosis seem to hold for the major spiral so far as shortening is concerned (PÄTAU 1939). They enable us to assume a further reduction to a fraction of the order of one-quarter, which accounts for spiralisation coefficients up to the value of 18 found in *Tulipa*.

An important corollary of this principle of the coiling of a thread of constant diameter is that the chromosomes should pass through stages differing in their internal stability under chemical treatment or mechanical tension. These stages should be of three distinct kinds in necessary

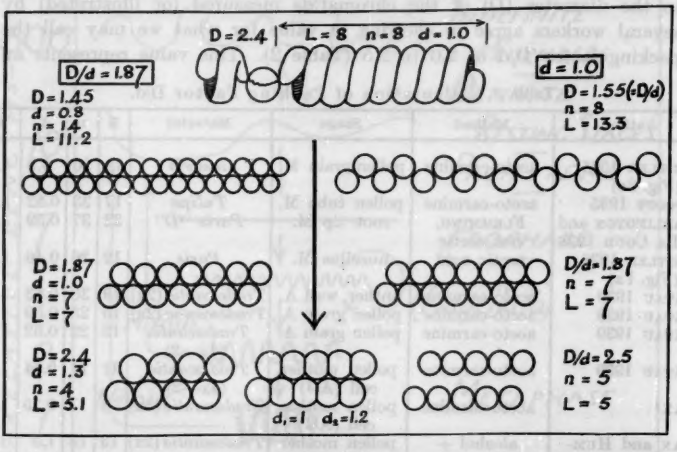

Fig. 2. Diagram to show various possible packing properties of the chromosome during spiralisation. *Above*, a model of a chromatid at anaphase with spherical centromere and left-handed coiling. D, diameter of chromatid; d, diameter of chromosome thread or of the region occupied by it; n, number of coils in a given length L. *Left*, development with close packing and a constant ratio D/d. *Right*, development with a constant value d, passing from an open spiral to a hollow spiral. *Middle, below*, the intermediate type with a compressed thread, probably most compatible with observation and with the model.

succession: *open, close* and *hollow* (Fig. 2). The open or loose spiral should not always be optically homogeneous. It should perhaps be more readily visible than the compact spiral. As BARBER points out (1939) and as BELAR's photographs seem to show, this is probably true of one stage of prophase in mitosis. Further the hollow spiral should be more easily unravelled than the compact spiral. At the second meiotic division, especially in the embryo-sacs of plants (DARLINGTON and LA COUR, unpublished) an unstable condition of the major spiral shows itself by some of the chromosomes having a major spiral in part of their length while others have none at all.

The low stability of the major spiral at first metaphase of meiosis is shown in four ways; by its being more readily undone than the minor

spiral by ammonia and heat treatment; by its not being developed in small fragments (DARLINGTON 1937b); by its loss during interphase when the minor spiral is often retained; and by its appearing as an open spiral in chromatids under tension, just as in *Aggregata* with a very low degree of spiralisation the mitotic anaphase chromosomes, which perhaps are in an open spiral stage, may show their spiral under tension. In all such cases we must enquire whether instability depends on the amplitude or wave length of the spiral *per se* or on its method of packing. In the first meiotic division it is clear that both are different from their values at mitosis. It seems that the packing factor (D/d) is lower at meiosis than at mitosis in *Tradescantia* (PĂTAU 1939).

A number of other considerations warn us that this proximate solution is only the beginning of the problem. Where, in aberrant cells at the first metaphase of meiosis, the chromosome is less extended, there are more major coils than usual (DARLINGTON 1937b). We must therefore suppose, as we should expect *a priori*, that contraction goes along with a reduction of the number of coils and an increase in their wave length. If this is so the major spirals can be supposed to have arisen as minor coils at an earlier stage and to have developed from these minor coils by a continuous process. In other words the minor coils can develop inside and after the major coils. The diameter of the thread which is developing these coils would then increase as in the diagram to the left of Fig. 2. Such a system would permit of the even close packing represented in this figure at all stages of development.

Now we have no certainty that the beginning of this stage could be detected in metaphase chromosomes. Every gradation should be possible between a characteristic single coiling and a double coiling. And finally underneath all these changes there must be a molecular rearrangement, a compensating molecular spiral to allow the external changes to take place without external torsion. These changes are no doubt associated with hydration changes, as KUWADA has pointed out (1937), changes which are as yet unmeasured.

This theory of continuous development is favoured by the continuous gradation in contraction to be found in metaphase chromosomes within the same species — for example in variable pollen mother-cells and pollen grains in *Fritillaria* and *Tradescantia*, *Tulipa* as well as in spermatocytes of *Stauroderus* (DARLINGTON, 1936, 1937b, UPCOTT unpub.). The alternative view that the major spiral is superimposed on the minor spiral during meiosis is supported by PĂTAU's measurements showing a constant length in the variously coiled chromosome thread at different stages of mitosis and meiosis. It might also seem to be favoured by the corrugated outline of late diplotone chromosomes in plants. This may however be due to the unstable packing stage already referred to.

The evidence of sub microscopic spiralisation is now available from the second method of analysis. SCHMIDT (1937, 1938) finds by double refraction of polarised light that in the salivary gland chromosomes with 300/1 extension the doubly refractive particles are arranged lengthwise in the fibre, in the mitotic chromosomes crosswise and in the meiotic chromosomes again lengthwise. This supports the direct observation of single coiling in the mitotic chromosomes and double coiling in the meiotic. But KUWADA and NAKAMURA also find evidence in the same way of double coiling in certain pollen grain mitoses. Our suspicion that the visible coiling may not always reflect the essential internal relationship is thus strengthened.

What then, it will be asked, is the relationship between the extended salivary gland bundles and the pachytene threads which are presumably 20 or 30 times shorter? The pachytene chromosomes can scarcely be supposed to have any important coiling of the essential gene structures, since these must be at this stage responsible for the specific two-by-two attractions of threads. The chromomeres however by their nucleic acid content are alone responsible, as PÄTAU (1939) points out, for the doubly refractive properties of the chromosome, and since CASPERSSON (1936) has shown that the nucleic acid is confined to the chromomeres in pachytene and salivary gland chromosomes, the problem is simplified. We can consider the extension of the chromosome between chromomeres to be responsible for the increase of length when the salivary chromosomes are compared with pachytene. Such an extension would be of the same order as that occurring in the denaturation of protein, whose structure, whether a self-compensating spiral or zig-zag, is for the moment irrelevant. Its result would be that the apparent pachytene chromomeres would correspond to groups of salivary chromomeres. And further that the considerable changes observed in the lengths of chromosomes during pachytene, extension in *Lilium*, contraction in *Chorthippus*, would be compatible with the absence of any spiral structure such as could be detected with polarised light.

Summary.

The observations of lengths of chromosomes in salivary glands at pachytene and in mitotic and meiotic metaphases are consistent with the observed coiling of the chromosomes in mitosis and meiosis in plants, animals and Protista, and with the doubly-refractive properties of the chromomeres and chromosomes, on various possible assumptions with regard to the packing of the spiral and the internal changes correlated with spiralisation.

Further measurements of the kind that are brought together here, as well as advances from the chemical side, should make it possible

to discriminate between these assumptions. It seems likely that different states of packing are responsible for the different degrees of mechanical and chemical stability at various stages in the development of spirals. And further that spirals microscopically visible at metaphase may be beyond the range of visibility at an earlier stage in their development.

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References.

Barber, H. N.: The Rate of Movement of Chromosomes on the Spindle I. Chromosoma **1** (1939). — **Belar, K.:** Der Formwechsel der Protistenkerne. Erg. Zool. **6**, 235—652 (1926). — **Belling, J.:** The ultimate chromomeres of *Lilium* and *Aloë* with regard to the number of genes. Univ. California Publ. Bot. **14**, 307—318 (1928a). — Contraction of chromosomes during maturation divisions in *Lilium* and other plants. Univ. California Publ. Bot. **14**, 335—343 (1928b). — Chromomeres of liliaceous plants. Univ. Californiia Publ. Bot. **16**, 153—170 (1931). — **Bridges, C. B.:** Salivary chromosome maps. J. Hered. **26**, 60—64 (1935). — **Caspersson, T.:** Über den chemischen Aufbau der Strukturen des Zellkernes. Skand. Arch. Physiol. (Berl. u. Lpz.) **73**, Suppl. 8 (1936). — **Cleveland, L. R.:** Longitudinal and transverse division in two closely related flagellates. Biol. Bull. Mar. biol. Labor. Wood's Hole **74**, 1—24 (1938). — **Dark, S. O. S.:** Chromosome studies in the Scilleae, II. J. Genet. **29**, 85—98 (1934). — **Darlington, C. D.:** The origin and behaviour of chiasmata, V. *Chorthippus elegans*. Biol. Bull. Mar. biol. Labor. Wood's Hole **63**, 357—367 (1932). Meiosis in *Agapanthus* and *Kniphofia*. Cytologia **4**, 229—240 (1933). — Anomalous chromosome pairing in the male *Drosophila pseudo-obscura*. Genetics **19**, 95—118 (1934a). — The origin and behaviour of chiasmata, VII—. *Zea Mays*. Z. Abstammgslehre **67**, 96—114 (1934b). — Crossing-over and its mechanical relationships in *Chorthippus* und *Stauroderus*. J. Genet. **33**, 465—500 (1936). — Recent Advances in Cytology, 2nd. Ed. London (1937a). — Chromosome Behaviour and Structural Hybridity in the Tradescantiae II. J. Genet. **35**, 259—280 (1937b). — **Darlington, C. D. and L. La Cour:** Differential reactivity of the chromosomes. Ann. Bot. **2**, 615—625 (1938). — **Duryee, W. R.:** Isolation of nuclei and non-mitotic chromosome pairs from frog eggs. Arch. exper. Zellforsch. **19**, 171—176 (1937). — **Geitler, L.:** Der Spiralbau somatischer Chromosomen. Z. Zellforsch. **23**, 514 bis 521 (1935). — Weitere Untersuchungen über den Spiralbau somatischer Chromosomen. Z. Zellforsch. 28, 305—309 (1938). — **Koller, P. C.:** The origin and behaviour of chiasmata, XI. *Dasyurus* and *Sacrophilus*. Cytologia **7**, 82—103 (1936). **Koltzoff, N. K.:** The Structure of the Chromosomes and their Participation in Cell-Metabolism. Biol. J. Moscow **7**, 3—46 (1938). — **Kuwada, Y.:** The hydration and dehydration phenomena in mitosis. Cytologia, Fujii Jub. Vol. **1937**, 389 bis 402. — **Kuwada, Y.** and **T. Nakamura:** Behaviour of chromonemata in mitosis. I. Observation of pollen mother cells in *Tradescantia reflexa*. Mem. Coll. Sci. Kyoto B **9**, 129—139 (1933). — Behaviour of chromonemata in mitosis. IV. Double refraction of chromosomes in *Tradescantia reflexa*. Cytologia **6**, 78—86 (1934). — **Makino, S.:** The spiral structure of chromosomes in the meiotic divisions of *Podisma* (Orthoptera). J. Coll. Sci. Hokkaido VI **5**, 29—40

(1936). — **Marquardt, H.:** Die Röntgenpathologie des Mitose I, II. Z. Bot. **32**, 401—482 (1938). — **Matsuura, H.:** On the number of spiral gyres in the chromonemata. Jap. J. of Bot. **19**, 143—149 (1934). — **McClintock, B.:** A method for making aceto-carmin smears permanent. Stain Technol. **4**, 53—56 (1929). — The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea Mays*. Z. Zellforsch. **19**, 191—237 (1933). — **Nakamura, T.:** Double refraction of the chromosomes in paraffin sections. Cytologia, Fujii Jub. Vol. **1937**, 482—493. — **Pätau, K.:** SAT-Chromosom und Spiralstruktur der Chromosomen der extrakapsulären Körper (*Merodinium* sp.) von *Collozoum inerme* MÜLLER Cytologia Fujii Jub. Vol. **1937**, 667—680. — Spiralstruktur der Chromosomen von *Tradescantia*. Chromosoma **1** (1939). — **Rückert, J.:** Zur Entwicklungsgeschichte des Ovarialeies bei Selachiern. Anat. Anz. **1**, 107—158 (1892). — **Sax, K.** and **L. M. Humphrey:** Structure of meiotic chromosomes in microsporogenesis of *Tradescantia*. Bot. Gaz. **96**, 353—362 (1934). — **Sax, H. J.** and **K. Sax:** Chromosome structure and behaviour in mitosis and meiosis. J. Arnold Arbor. **16**, 423—439 (**1935**). — **Schmidt, W. L.:** Doppelbrechung und Röntgendiagramm der Nucleinsäure und Feinbau der Chromosomen. Naturwiss. **24**, 413 (1938). — **Straub, J.:** Neuere karyologische Probleme und Ergebnisse. Z. Bot. **33**, 65—126 (1938). — **Upcott, M.:** The mechanics of mitosis in the pollen-tube of *Tulipa*. Proc. roy. Soc. Lond. B **121**, 207—220 (1936). — **White, M. J. D.:** The chromosomes of the domestic chicken. J. Genet. **26**, 315—350 (1932).

(John Innes Horticultural Institution Merton.)

THE RATE OF MOVEMENT OF CHROMOSOMES ON THE SPINDLE.

By

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With 8 figures in the text.

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Contents.

I. Introduction.

In order to understand the forces at work or the materials concerned in chromosome movement and cell division it is of the first importance to know the speeds of movement and how far these speeds are dependent on external conditions such as temperature. In all the extensive observations of these processes that have been made, the detailed information needed for this purpose seems to have been left unrecorded.

The object of the present study has been to make original measurements of the rates of anaphase movement of chromosomes at mitosis and to collect parallel data from certain published records that have been made for other purposes.

II. Material and Methods.

The material used was *Tradescantia virginiana*. The tetraploid ($4x = 24$) has a longer flowering period (May—October) than the diploid and was used in preference to it. Dividing cells are easily obtained in both young staminal hairs and petals (Belar 1929b). Divisions go on over a fairly long period in the development of the flower, but they are most abundant in flowers which have just completed meiosis in the anthers. To make the material as homogeneous as possible, divisions were obtained from flowers at this stage of development.

Liquid paraffin was used as a mounting medium for the microscopical investigation of the living cells. Isotonic sugar solutions and physiological saline-sugar solutions are not so satisfactory, for a large proportion of the cells always die and it is difficult to keep the solutions clean. Liquid paraffin on the other hand appears to have no effect on the living cells, and unless they have been damaged in dissection mitosis goes on quite normally for at least 12 hours. A large proportion of the early prophases will go right through to telophase. A further advantage of liquid paraffin is that it penetrates between the cells and air-bubbles are easily avoided.

Mitosis ceases completely in preparations which are 24 hours old. The cells are not dead, and protoplasmic streaming may be quite vigorous after 14 days or longer in liquid paraffin under a cover slip. It is difficult to see why mitosis should cease so rapidly. It is probably not due to oxygen lack; oxygen is necessary for protoplasmic streaming and its solubility in liquid paraffin is 4—5 times that in water. The same stoppage occurs in sugar solutions, although here it is difficult to prevent contamination with micro-organisms. Divisions in the staminal hairs also cease in inflorescences which are cut and placed in water for 4—5 days. Attempts to culture meristematic parts of flowers in media which have proved excellent for root-tips, e. g. WHITE's (1936) or BONNER and ADDICOTT's (1937) media, show a similar series of changes. Divisions cease within a week or ten days, although the tissues may remain alive for periods up to 3 months (LA RUE 1938, BARBER unpublished). The nuclei become spherical and decrease in size. Granules disappear from the cytoplasm and the cells appear "starved". Cessation of growth appears to be due either to difficulties of absorbing a sufficient amount of food material or to lack of some accessory growth substance.

Photography. Measurement of the rates of movement of chromosomes was made by photographing the cell at definite intervals. By direct observation it is impossible to get accurate results. The photography was carried out with a camera designed by Mr. H. C. OSTERSTOCK, using 35 mm. ciné film. Exposures of about 4 secs. were made at intervals of $^1/_2$ or 1 minute. Over-heating by the lamp was prevented by means of a tank filled with a solution of ferrous ammonium sulphate (BELAR, l. c.). Moderate stopping down of the substage iris is essential for good definition. A green filter was inserted, though it proves to give little if any increase in contrast.

Sixteen mm. ciné films were also taken on a ciné camera designed for photomicrography by Dr. A. F. W. HUGHES of the Strangeways Laboratory, Cambridge. I am greatly indebted to Dr. HUGHES for the help he gave me in using the camera, and also to Dr. H. B. FELL for permission to work at the Laboratory. I wish also to thank Dr. C. S. HANES of the Low Temperature Station, Cambridge, for allowing me to use constant temperature chambers at the Station. Films were taken at three temperatures.

III. Prophase and metaphase in *Tradescantia*.

Mitosis as seen in living *Tradescantia* has been well described by BELAR (1929 b). The resting nucleus is of the "solid" type uniformly filled

with chromosomes (MANTON 1936), and shows a definite structure of intertwined threads, with one or more nucleoli which remain in the same position in the resting nucleus for long periods (cf. GRAY 1931). The resting nuclei are not stationary in the cell but move about as the protoplasm streams. The ends of the cells of the staminal hairs are filled with 2 or more fairly large vacuoles, which during the resting stage and prophase show continual movement. In films taken at 8 exposures per minute and projected at 16 per second these protoplasmic movements are of course exaggerated and appear as a vigorous "boiling".

Prophase begins with a change in texture of the resting nucleus. Spirals appear, which may be visible for half an hour or more. They then condense into thicker, optically homogeneous pairs of chromatids which show no trace of spiral structure except for the relic coils from the preceding division. The rest of the prophase lasts 3—4 hours, when we reach the very characteristic late prophase with the chromosomes fully contracted and lying parallel with all the centromeres (spindle attachments) at one end of the nucleus. This arrangement is derived directly from the previous telophase. At this stage the chromosomes may not fill the nucleus completely. Definite gaps are often visible at each end of the nucleus, filled with transparent material and sharply shut off from the granular cytoplasm by the nuclear membrane. Shortly after this the nuclear membrane disappears, and the centromeres of the chromosomes are pushed down on tc the metaphase plate (congression, DARLINGTON 1936). The distance moved is usually about 10μ and at room temperature (20—22° C.) congression takes 15—20 minutes. The average rate of movement is about 0.5μ/minute.

During congression the protoplasmic movement gradually slows down, and at metaphase and early anaphase the "boiling" of the protoplasm as seen in the films of prophase stops completely. There may be slow swaying movements in the chromosome arms.

Full metaphase lasts 15—25 minutes (see Table 1).

IV. The anaphase movement in *Tradescantia*.

The transition from metaphase to anaphase is abrupt. The metaphase plate quite suddenly becomes less distinct as the chromatid attraction lapses, and the two chromatids of each chromosome separate slightly. The centromeres gradually draw apart. Fig. 2 shows the distance between the two groups of centromeres plotted against time, the distances being measured from photographs. It is impossible to follow one pair of centromeres over more than 3 or 4 successive photographs, and the distances plotted represent the mean of the distances between as many pairs of centromeres as are visible on each photograph.

The centromeres reach their maximum velocity within 1—2 minutes of the beginning of anaphase. At 20° C. the maximum velocity of the

Table 1.

Position of cell in hair	Dimensions of cell (μ)	Duration of metaphase (mins.)	Maximum separation at anaphase		Velocity of chromosomes		Time from end of anaphase movement to cell plate formation
			Time taken (mins.)	Distance attained (μ)	Mean μ/min.	Max. μ/min.	
Temperature 35° C.							
1	76 × 17	20	21	31.5	0.75	1.16	2
2	62 × 20	—	24	35.0	0.73	1.23	3
Temperature 20—22° C.							
2	57 × 16	20	23	37.5	0.82	1.32	11
1	65 × 18	15	27	29.0	0.54	1.12	—
1	56 × 15	15	25	33.0	0.66	1.35	7
1	59 × 16	—	24	27.5	0.57	0.99	10
1	70 × 14	20	21	33.0	0.79	—	7
Petal	40 × 21	—	19	25.0	0.66	—	4
Petal	41 × 16	—	16.5	27.5	0.84	—	9.5
Temperature 10° C.							
3	42 × 24	120	63	31.0	0.26	0.29	41
1	69 × 18	50	76	40.0	0.24	0.26	19
1	75 × 16	150	68	41.0	0.30	0.35	30
1	60 × 17	175	84	37.0	0.22	—	26
1	64 × 16	—	80	36.0	0.23	—	40

centromeres relative to one another is between 2.0 and 2.6 μ per minute, or half this velocity relative to their starting place. The velocity gradually

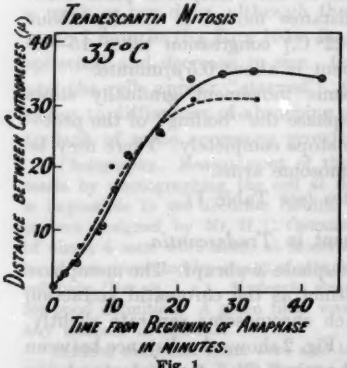

Fig. 1.

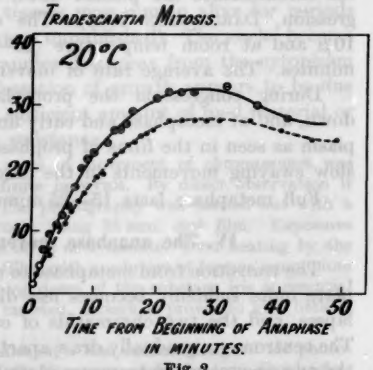

Fig. 2.

Fig. 1. Anaphase curves for two staminal hair cells of *Tradescantia virginiana*, temp. 35° C. In all the *Tradescantia* curves the centromeres have been assumed to be 1 μ apart at the beginning of anaphase. It is difficult to obtain accurate measurements at early anaphase.

Fig. 2. Anaphase curves for *Tradescantia*, temp. 20° C.

decreases until movement ceases at a maximum distance of about 30 μ. Further separation is apparently prevented by the vacuoles at

the end of the cell, and not by the cell walls, so that there is no very close relation between the length of the cell and the maximum separation. The two anaphase nuclei may bulge into the vacuoles in very short cells. The anaphase movement takes about 25 minutes to complete (mean velocity over the whole distance = 0.70 μ/minute) and after movement has ceased the chromosomes contract to form the telophase nucleus, the minimum length attained being about 2/3 metaphase length.

During metaphase and anaphase it is extremely difficult to make out any definite spindle in the living cell. Whilst the chromosomes are contracting to form the telophase nuclei, the whole cell undergoes very

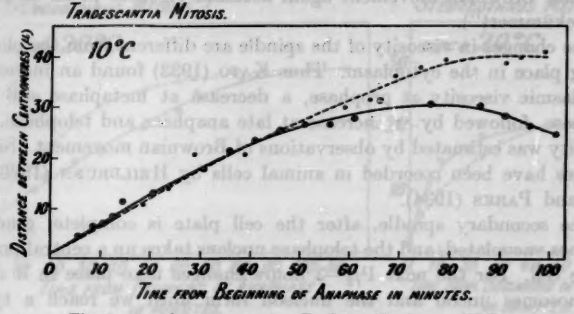

Fig. 3. Anaphase curves for *Tradescantia*, temp. 10° C.

vigorous cytoplasmic movements. Granules appear to flow down from the poles and penetrate between the two telophase nuclei right into the spindle region. They may show vigorous Brownian movement. This granular stage lasts only a short time (5—10 minutes), and then a narrow secondary spindle (Darlington and Thomas 1937) ("phragmoplast") connecting the two telophase nuclei appears. It rapidly grows in width and contracts in length, drawing the two telophase nuclei nearer to each other. The cell plate appears in the centre of it and rapidly grows across the cell. (Cell plate formation is usually complete 40 minutes after the beginning of anaphase and takes on the average 10 minutes from its first appearance to divide the cell completely.)

These observations prove that the cell-plate forming spindle is of independent origin from the late anaphase spindle (Becker 1938). It appears to be more viscous, since the granules in it do not show Brownian movement to the same extent as at late anaphase. Wada's (1935) microdissection experiments lead to a similar conclusion. The late anaphase spindle after chromosome movement has ceased, seems to be fluid — for example Wada found it possible to move one anaphase group of chromosomes by sticking a needle into it, without moving the

other. But later on, as the chromosomes contract, the spindle shows a greater viscosity and rigidity.

The metaphase spindle is presumably a fairly rigid body (CHAMBERS and SANDS 1923). The anaphase movement seems to involve the secretion of fluid or the dissolution of the spindle between the separating chromosomes. BELAR's observations (1926) on Brownian movement of granules inside the spindle in *Tradescantia* are interesting in this connection. During the development of the spindle he observed unrestricted Brownian movement. But at metaphase it was restricted in directions transverse to the spindle, though vigorous in channels along the spindle. At late anaphase Brownian movement again becomes vigorous in all directions („unbekümmert").

The changes in viscosity of the spindle are different from the changes taking place in the cytoplasm. Thus KATO (1933) found an increase in cytoplasmic viscosity at prophase, a decrease at metaphase and early anaphase, followed by an increase at late anaphase and telophase. The viscosity was estimated by observations of Brownian movement. Similar changes have been recorded in animal cells by HEILBRUNN (1926) and FRY and PARKS (1934).

The secondary spindle, after the cell plate is complete, gradually becomes vacuolated, and the telophase nucleus takes up a central position in the cell. For the next $1^1/_2$—3 hours changes take place in it as the chromosomes uncoil and the nucleoli form until we reach a typical resting structure. All these changes may be considered as telophase.

V. Anaphase movement in other types of cell.

1. Mitosis in human and chick fibroblasts.

Through the courtesy of Dr A. HUGHES and Dr J. BLAND of the Strangeways Laboratory I have been able to examine films of mitosis in chick and human fibroblasts. Unfortunately in most of these cases the absolute magnification and timing of the successive exposures are not known. But it is possible to obtain arbitrary curves showing the type of anaphase separation. They are similar to those of *Tradescantia* except that the velocity of the centromeres tends to remain constant over most of the distance travelled, and only falls off appreciably at the end of the movement. Division in these cells is by constriction, and consequently we do not get the coming together of the two telophase nuclei which we saw in *Tradescantia*. The changes which take place in the spindle during anaphase seem to be far simpler in this type of cell. It undergoes a gradual stretching as the centromeres move apart, this stretching, according to the „Stemmkörper" theory of BELAR, providing most of the propulsive force. It does not seem to become less viscous or rigid in these cells as anaphase progresses. Absolute values for the rates of movement of the chromosomes in these cells would be very

interesting. The time of anaphase in chick fibroblasts has been measured by numerous authors (STRANGEWAYS 1922, LEWIS 1917, BUCCIANTE 1927). The mean time is about 2.5 minutes at 39° C. (minimum 1 minute, maximum 4—5 minutes). So far as I have been able to find, the maximum distance apart reached has not been measured in the living cell. But from fixed cells in preparations lent to me by Dr. W. JACOBSON of the Strangeways Laboratory the distance between the two nuclei at telophase is about 13 μ (mean of ten cells). Thus the absolute velocity of chick chromosomes at 39° C. is 2.6 μ/minute (minimum 1.6 μ/ minute,

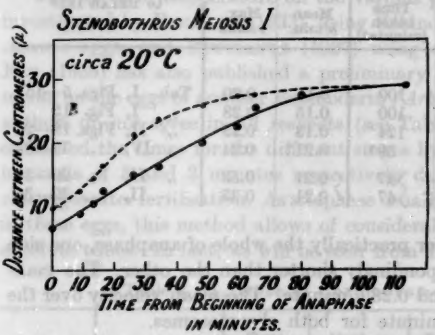

Fig. 4.

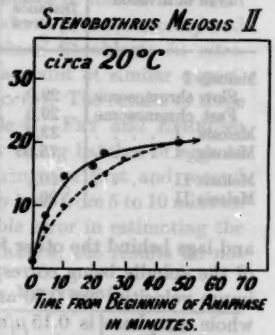

Fig. 5.

Fig. 4. Anaphase I curves for two bivalents in the same cell of *Stenobothrus*. The lower curve is that for the bivalent with interstitial chiasmata. The upper has only a single terminal chiasma.

Fig. 5. Anaphase II curves for *Stenobothrus*. The curves are for two sister cells. The centromeres are assumed to be 1 μ apart at the beginning.

maximum 6.5 μ/minute). The rates are appreciably higher than those in either *Tradescantia* at 35°C. or *Stenobothrus* at 20°C. (see below).

2. *Meiosis in the spermatocytes of Stenobothrus.*

The anaphase movement in *Stenobothrus* has been well analysed by BELAR (1929), and it was on this material that he founded his „Stemmkörper" theory of the mechanism of anaphase. He gives no data as to the absolute rates of movement of chromosomes, but from his photographs (Plates I and II, BELAR 1929a) we can measure the distances travelled by the chromosomes in given times. The material is better in one respect than the *Tradescantia* and tissue culture mitoses in that separate chromosomes are easily distinguishable right through the anaphase.

Fig. 4 shows the distances between the centromeres of two bivalents in a *Stenobothrus* primary spermatocyte plotted against time. The bivalents are about the same size, but differ in structure. The first has one

interstitial chiasma in the longer arm and two — one terminal — in the shorter arm, whilst the other has a single terminal chiasma in the shorter arm and none in the longer. We might expect that the longer the chromatids that have had to separate distal to a chiasma in a bivalent, the slower would be the commencement of anaphase movement. This seems to be the case. The bivalent with the interstitial chiasmata starts more slowly

Table 2.

Type of division	Maximum separation at anaphase: Distance attained μ	Maximum separation at anaphase: Time taken (minutes)	Chromosome velocities: Mean μ/min.	Chromosome velocities: Max. μ/min.	Reference to Belar 1929
Meiosis I					
Slow chromosome	29.0	100	0.15	0.20	Tab. I, Figs. 5—15
Fast chromosome	29.0	100	0.15	0.28	„ I, Fig. 15
Meiosis I	32.0	124	0.13	0.23	„ II, Figs. 18—24
Meiosis I	25.0?	59?	0.21?	0.24	„ IV, „ 73—82
Meiosis II	>20	>47	<0.21	0.53	„ II, „ 29—36
Meiosis II	>20	>47	<0.21	0.33	„ II, „ 29—36

and lags behind the other for practically the whole of anaphase, one side of the spindle being correspondingly shorter than the other. The maximum velocities are 0.20 and 0.28 μ/minute. The mean velocity over the whole anaphase is 0.15 μ/minute for both chromosomes.

Cell division is by constriction as in the fibroblasts, and there is no coming together of the two daughter nuclei at telophase.

Similar data for the second meiotic division are given in Table 2. Here there is no question of separating chromatids, so that the situation is simpler even than that of mitosis. The velocities are rather greater than at the first division. (Mean maximum velocity = 0.43 μ/minute),

Table 3. Chromosome Velocities in Different Organisms.

Organism	Movement at	Temp. °C.	Mean distance travelled (μ)	Mean velocity (μ/min.)	Max. velocity (μ/min.)	Source
Tradescantia virginiana 4 x	Mitosis, congression	10	10	0.19	—	Own data
„ „	„ „	20	10	0.50	—	„ „
„ „	Mitosis, anaphase	10	16.5	0.25	0.30	„ „
„ „	„ „	20	16.5	0.70	1.20	„ „
„ „	„ „	35	16.5	0.74	1.20	„ „
Gallus domesticus	„ „	21	6.5	0.28	—	Buccianti 1927 and own data (see text)
„ „	„ „	41	6.5	3.68	—	„ „
„ „	„ „	45	6.5	3.25	—	„ „
Stenobothrus lineatus	Meiosis I, anaphase	ca. 20	15	0.14	0.24	Belar 1929
„ „	Meiosis II, anaphase	ca. 20	14	0.21	0.43	Belar 1929

and the curves are of a rather different shape from the 1st division curves. We may explain the lower velocity and flattening of the earlier parts of the anaphase curve at the 1st meiotic division by its two special properties: first the resistance of the chromatids distal to chiasmata to separation, and secondly to the centromeres, whose mutual repulsions initiate movement, being a greater distance apart at the beginning of first anaphase than at the second. They do not get a flying start.

VI. Temperature control of the anaphase movement.

The effect of temperature on the various phases of mitosis has been investigated by Ephrussi (1933), using Echinoderm *(Paracentrotus)* and *Ascaris* eggs, and Buccianti (1927) using chick fibroblasts *in vitro*. Fry (1936) has also published a preliminary account of similar experiments on the eggs of another Echinoderm *(Arbacia)*. The results of these authors do not agree in all respects (see Table 4). Fry and Ephrussi estimated the times for the different stages by fixing batches of eggs at intervals of 5 and 2 minutes respectively during the first and second cleavages after fertilisation. As anaphase usually lasts from 5 to 10 minutes in these eggs, this method allows of considerable error in estimating the absolute times. In fact, as will be seen from Table 4, the results do not

Table 4. Q_{10} Rate of Chromosome Movement.

Author	Organism	Type of cell	Nuclear Stage	Temperature range (° C)	Q_{10} Low temp.	Q_{10} High temp.
			(1) Observations on Fixed Material.			
Ephrussi, 1933	*Paracentrotus* (Echinoderm)	Egg	Prophase	12.7—18.5—26	5.88	2.31
			Metaphase		3.10	1.00
			Anaphase		1.00	1.71
Ephrussi, 1933	*Ascaris* (Nematode)	Egg	Prophase	24—34	1.66	
			Metaphase		1.22	
			Anaphase		2.00	
Fry, 1936	*Arbacia* (Echinoderm)	Egg	Prophase	15—20—25	6.97	2.79
			Metaphase		1.73	4.00
			Anaphase		4.50	1.24
			(2) Observations of Living Cells.			
Buccianti, 1927	*Gallus* (Aves)	Fibroblast	Anaphase	21—45	10.0[1]	0.5[1]
			Cleavage furrow		15.0[1]	0.5[1]
Barber, 1939	*Tradescantia* (Monocot.)	Staminal hair	Metaphase	10—20—35	8.20	ca. 1.0
			Anaphase		2.33	1.05

[1] See text.

agree with one another and it seems unlikely that such differences can be explained by the two investigators having worked with different species of Echinoderm.

Bucciante's observations are by far the most complete. He timed 1250 living cells over anaphase and the cleavage furrow at temperatures ranging from 21° C. to 45° C., at intervals of 1° C. Fig. 6 gives a graphical representation of his results. The reciprocal of the mean time taken over anaphase and for the cleavage furrow to constrict the cell completely are plotted against temperature. Assuming that change of temperature has no effect on distance travelled at anaphase, 1/T gives a measure of the speed of movement of the chromosomes. We can make an estimate of

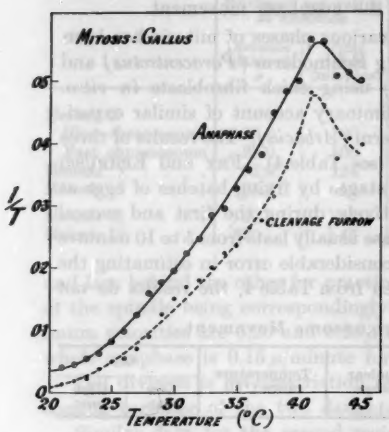

Fig. 6.

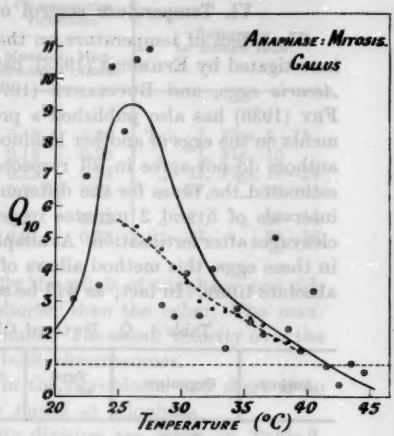

Fig. 7.

Fig. 6. Graphs of rate of chromosome movement and rate of growth of cleavage furrow against temperature in the chick.

Fig. 7. Q_{10} values for rate of chromosome movement in the chick plotted against temperature. The solid points represent the values as calculated by *Bucciante*; the others are calculated by the method here described (see Appendix).

the absolute velocity as we know the length of the telophase spindle to be on the average 13 μ. Thus at 21° C. the absolute velocity is of the order of 0.3 μ/minute whereas at 41° C., the optimum temperature, it is 3.64 μ/minute, falling to 3.25 μ/minute at 45° C., the maximum temperature at which cell division in the chick occurs.

I have made a preliminary investigation of the effect of temperature on the rate of chromosome movement in *Tradescantia* staminal hairs. Films were made at temperatures of 10° C., room temperature (20—22° C.) and 35° C.). The apparatus was set up in a constant temperature chamber and cells at late prophase selected for photography, so that at least one hour elapsed before the onset of anaphase. Figs 1, 2, 3 and Table 1 show the type of anaphase curves obtained at the three temperatures, and in

Fig. 8 the maximum and mean velocities of the chromosomes at anaphase are plotted against temperature. The curves are drawn through the mean values for the three temperatures. In some cases it was impossible to get a good estimate of the maximum velocity. The time taken over anaphase shows, of course, changes corresponding to the velocity changes.

Q_{10} values calculated from the data are given in Table 4. The Q_{10} for the maximum velocity is greater than for the mean. Thus for the temperature interval 10—20° C. Q_{10} for maximum anaphase velocity is 4.78; for mean anaphase velocity $Q_{10} = 2.33$.

The data for the velocity of chromosome movement during congression on the metaphase plate are not so complete as for anaphase. The velocity is much slower at 10°C than at 20° C. In one cell at 10°C., the chromosomes moved about 9.0 μ in 48 minutes — a mean velocity of 0.19 μ/minute. At 20° C., as we have seen above, the velocity is 0.5μ/min. No data are available for 35° C. Q_{10} over the temperature interval 10—20° C. is thus 2.6 and of the same order of magnitude as the anaphase Q_{10}.

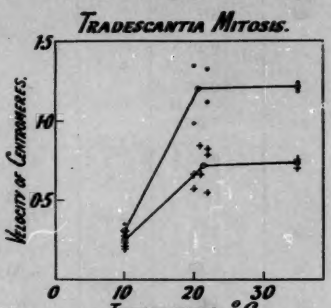

Fig. 8. Velocity-temperature graphs for *Tradescantia* anaphase. The black points represent the maximum velocity of the centromeres, the crosses the mean velocities. In both cases the lines are drawn through the mean values for the several cells measured.

It is impossible to say at present whether these results agree with BUCCIANTE's on the chick. BUCCIANTE gives a table of Q_{10}s calculated from his data (Table 4 and 5, p. 10, BUCCIANTE 1927). They apparently show a gradual decrease in Q_{10} the higher the temperature interval considered, the Q_{10} never falling below unity. The Q_{10} for *Tradescantia* over the temperature interval 10—20° C. is 2.33 for the mean anaphase velocity. For the interval 20—35° C. $Q_{10} = 1.05$, so that on this reckoning they do agree. But as we have seen above, BUCCIANTE's curve shows a definite optimum temperature at which chromosome velocity is at a maximum. His method of calculating Q_{10} provides a very inefficient measure of the effect of temperature (see appendix).

If R_T and R_{T+10} are the rates of a process at temperatures T° C. and (T + 10)° C. then the Q_{10} is given by $\frac{R_{T+10}}{R_T}$.

We can show the variations of velocity in terms of Q_{10} only if we make the temperature interval over which we calculate smaller than 10° C. BUCCIANTE's experiments were done at intervals of 1° C. We can, therefore, calculate a Q_{10} value for each of these intervals of 1° C. (see Appendix).

Table 5. Q_{10} values for time of anaphase and cleavage furrow in the chick.

Temp. °C	Duration of anaphase (mins.)	Q_{10}	Bucciante's Q_{10}	Duration of cleavage furrow (mins.)	Q_{10}	Bucciante's Q_{10}
21	24.37			71.57		
		3.11			33.96	
22	21.75			50.30		
		7.01			6.34	
23	17.90			41.82		
		3.55			124.5	
24	15.77			25.82		
		10.50			14.93	
25	12.47			19.70		
		8.41			2.81	
26	10.08		5.45	17.77		12.20
		10.69			9.98	
27	7.95		5.43	14.12		9.06
		11.09			12.45	
28	6.25		5.09	10.97		8.44
		2.89			28.44	
29	5.62		4.66	7.85		5.80
		2.53			5.65	
30	5.12		4.15	6.60		4.50
		3.89			3.17	
31	4.47		3.60	5.88		4.69
		2.70			1.88	
32	4.05		3.03	5.52		3.90
		4.07			2.98	
33	3.52		2.79	4.95		3.46
		1.50			2.90	
34	3.38		2.71	4.45		2.83
		2.79			1.41	
35	3.05		2.55	4.30		2.75
		2.35			3.63	
36	2.80		2.50	3.78		2.84
		1.94			1.54	
37	2.62		2.27	3.62		2.54
		5.01			3.77	
38	2.23		1.78	3.17		1.86
		2.10			3.86	
39	2.07		1.73	2.77		1.81
		1.41			4.20	
40	2.00		1.52	2.40		1.70
		3.39			4.18	
41	1.77			2.08		
		0.95			0.65	
42	1.78			2.17		
		0.36			0.14	
43	1.97			2.65		
		1.01			2.18	
44	1.95			2.45		
		0.78			0.75	
45	2.00			2.52		

Table 5 gives the Q_{10} calculated by this method compared with Bucciante's. They are shown graphically in Fig. 7. It will be seen that Q_{10} increases rapidly to a maximum of about 10 at 26—27 and then

rapidly falls to more normal values and becomes less than 1 at 41—42° C. The actual values may not mean very much. Any experimental errors will be magnified, but the general trend of Q_{10} with temperature is unmistakable and gives a fairly good picture of the original data. The data for the time taken for the cleavage furrow to cut across the cell, given by BUCCIANTE, can be analysed similarly. They show very similar changes with temperature. The Q_{10} values and the fluctuations are rather greater.

The results agree completely with those of other authors (e. g. FAURÉ-FREMIET, 1925) on the effect of temperature on the velocity of segmentation of the eggs of *Ascaris* (cf. BUCCIANTE). Thus FAURÉ-FREMIET showed that in *Ascaris* eggs segmentation is most rapid at its normal temperature, 37° C., and falls off if the temperature is either raised or lowered from this point. We cannot, with FAURÉ-FREMIET, say that there is a negative Q_{10}, i. e —4.1, for the temperature interval 37—42° C. The Q_{10}, of course, falls below 1 above 37° C. (see Appendix).

Further data are needed before we can say whether, in other organisms, the rate of chromosome movement has a clear optimum temperature as in the chick. It is highly probable that when we have data over a closer series of temperatures in *Tradescantia* we shall find the same type of relationship holding.

VII. Viscosity control.

The chromosomes move, or appear to move, through a fairly viscous body — the spindle. It is possible that viscosity changes may be the primary agent in controlling chromosome velocity at different temperatures. On this assumption the velocity will be inversely proportional to the viscosity or to some power of it. We shall also have to consider the further possibilities that changes in viscosity may be secondary, i. e. viscosity affects the system indirectly by slowing down the supply of materials necessary for the growth of the spindle, or finally that its influence on the rate of chromosome movement is negligible. The effect of temperature on cytoplasmic viscosity has been investigated by several authors. Most of the work has been done on eggs of various marine invertebrates in the resting condition. As we have seen above (p. 38) the viscosity of cytoplasm is not constant during the nuclear division and also the viscosity changes in cytoplasm appear to be different from those occurring in the spindle. We cannot therefore infer that the effect of temperature is the same on cytoplasmic viscosity as on spindle viscosity. But no work, to my knowledge, has been done on the effect of different temperatures on the viscosity of the early anaphase spindle, with which we are now concerned. Examination of the results of the above authors may enable us to see whether the viscosity of the cytoplasm is affected in a way that will explain the effect of temperature on spindle movement.

The effect of temperature on cytoplasmic viscosity seems to vary according to the type of cell considered. Thus PANTIN (1924) found a gradual decrease of viscosity in *Nereis* (Annelida) eggs with rise in temperature, the Q_{10} falling off the higher the temperatures. Similar changes have been found in *Ascaris* eggs (FAURÉ-FREMIET 1925). HEILBRUNN (1925) on the other hand, showed that the viscosity of eggs of *Cumingia* (a Mollusc) varies in a totally different way with temperature. There is a definite maximum viscosity at about 15° C., with a rapid decrease on either side. Similar curves have been found for such diverse organisms as *Spirogyra* and the Myxophyta (BAAS-BECKING 1928). None of these organisms, except perhaps *Nereis*, show the type of viscosity change that we should expect if the measurements give a good indication of the change of spindle viscosity with temperature. In *Nereis*, however, the viscosity changes are far too small to explain the variations in chromosome velocity. We must assume either that the spindle viscosity is affected by temperature very differently from the cytoplasm, or that the effect of temperature is directly on the forces responsible for the movement, i. e. the centromere repulsion and the growth of the spindle.

It is, indeed, doubtful whether viscosity is at all relevant, as both long- and short-armed chromosomes show the same velocity at anaphase, as we can see in any cytological preparation of an organism with large size-differences in its chromosomes. The resistance to movement will vary as the surface, which may be ten or a hundred times as great in the long chromosomes as in the short. Moreover the rate of chromosome movement is so small compared with other cell movements, as we shall see, that resistance due to viscosity may be altogether unimportant. These considerations seem to show that the major part of anaphase movement must be due, as BELAR thought, to the growth of spindle between the two separating groups of chromosomes. The two groups at mitosis behave as two units only. It is through this process of spindle growth that anaphase movement is affected by temperature.

VIII. Comparison of anaphase movement with other types of cell movement.

Chromosomes move about the cell extremely slowly — the velocity is usually under $1\,\mu$ per minute, or about 1 millimetre per day. It is interesting to compare the rate with those of other bodies in the cell (see Table 6). Thus the streaming of granules of about 0.3—$0.4\,\mu$ diameter in the cytoplasm of mature *Tradescantia* hairs may attain a rate of $300\,\mu$ per minute. It is usually somewhat slower in cells which are capable of division. The disc-shaped chloroplasts of *Elodea* in the midrib cells of the leaf may move at a similar velocity (mean of four determinations gave $250\,\mu$ per minute). The chloroplasts are $4.5\,\mu \times 1.5\,\mu$ in size. Thus protoplasmic streaming can easily carry bodies of the same order of size as chromosomes along at rates far greater than

Table 6. Rates of movement of cells and cell organs.

	Organism	Type of movement	Size of body moved (μ)	Velocity of movement (μ/min.)
1	*Tradescantia*	Chromosome at anaphase	$18 \times 0.8 \times 0.8$	0.7
2	*Avena*	Growth in length of cell in coleoptile	—	less than 1
3	*Datura*	Growth in length of pollen tube	—	32—55
4	*Amoeba*	Amoeboid movement of whole cell	—	150
5	*Elodea*	Chloroplast in cytoplasmic stream	$4.5 \times 4.5 \times 1.5$	250
6	*Tradescantia*	Granule in cytoplasmic stream	0.4^3	300
7	*Mytilus*	End of cilium	$10 \times 0.1 \times 0.1$	22,500

those of the anaphase movement. It would be very useful to have accurate data on the influence of temperature on such streaming movements.

The rates of amoeboid and ciliary movement are again far greater than that of chromosome movement. Amoeboid movement, according to PANTIN (1924) is about 150 μ per minute. The protoplasm streaming inside a pseudopodium reaches greater velocities than this. The tip of a cilium may go through 22.5 mm. per minute (GRAY 1931). These movements are obviously of a very different nature from the anaphase movement.

Finally, the growth in length of individual cells may reach far higher velocities than the rate at which chromosomes move. Thus BUCHHOLZ, WILLIAMS and BLAKESLEE (1935) find that the pollen tubes of *Datura* may grow down the style at a rate of 32—55 μ per minute in different species. The rate must be much greater in some lilies with long styles. These are probably exceptional values for growth in length of a plant cell, although it is difficult to get comparable data for other types of cell. *Avena* coleoptiles grow at a maximum rate of 18 μ per minute (WENT and THIMANN 1937) during the extensional phase. How many cells are growing in length at this time is unknown, but it is probable that the rate of extensional growth of an individual cell is not very different from the rate of chromosome movement.

The energy required to move chromosomes about the cell cannot be very great compared with other processes requiring energy in the cell. It is of interest to note there is some evidence that during mitosis the oxidation-reduction potentials may change in certain types of cell. For example LUDFORD (1935) found that cancer cells decolorised methylene blue while undergoing mitosis. Observations on the output of CO_2 by Echinoderm eggs during segmentation, by VLÈS (1922) show an

increase just after cleavage. GRAY (1925) however was unable to obtain evidence of greater O_2 uptake during segmentation. In any case, in view of the many processes taking place simultaneously in dividing cells, it will be very difficult to attack the problems of chromosome dynamics from the energetic standpoint for some time to come. Deduction from experiments with non-living systems having an analogous structure may be capable of giving far more important results.

IX. Summary.

1. The rates of chromosome movement at anaphase in a Monocotyledonous plant, a bird and a grasshopper have been obtained. The velocities vary considerably in the different organisms (0.3 to 3.5 μ/min).

2. In *Tradescantia* the rate of movement of chromosomes on to the metaphase plate is slightly slower than the anaphase rate (0.5 μ/min. compared with 0.70 at 20° C.).

3. In both *Tradescantia* and *Stenobothrus* the acceleration of the chromosomes at the beginning of anaphase only takes $^1/_{10}$—$^1/_{12}$ of the total time taken over the anaphase movement. Thereafter there is a gradual deceleration. In human fibroblasts on the other hand the velocity is more constant, with a rapid deceleration at the end of the movement.

4. At meiosis, the presence of proximally situated chiasmata causes a marked diminution in velocity at first anaphase, owing to chromatid drag. At second anaphase the maximum velocity is greater, owing to the absence of chromatid drag and perhaps also to the centromeres being closer together and so initiating anaphase more efficiently.

5. The relationship of temperature to velocity at anaphase is not a direct one. In the chick there is a definite optimum temperature (41° C.) at which the anaphase velocity is greatest. Q_{10} values for both chick and *Tradescantia* decrease as the temperature is raised.

6. Chromosome velocities are $^1/_{100}$—$^1/_{200}$th the velocities of other commensurate particles in the cells, such as chloroplasts.

7. The viscosities of the spindle and cytoplasm are shown to be negligible in controlling chromosome movement by the equal velocities of chromosomes of different sizes. This view is supported by the observation that the effects of temperature on cytoplasmic viscosity are entirely inconsistent with its effect on anaphase movement. Temperature therefore acts on the movement of the chromosomes through properties of the spindle other than viscosity.

I with tc thank Dr. C. D. DARLINGTON for much help and criticism during this investigation.

Appendix: The Calculation of Q_{10}.

The calculation of Q_{10} for a biological process may present difficulties. Most biological processes such as growth, photosynthesis etc. show a typical Sachsian optimum curve. Such a curve is the resultant of two processes — (1) the accelerating effect of temperature on the process considered, (2) the disorganisation of the living system brought about by high temperatures. This is, in effect, the type of curve we obtain from BUCCIANTE's data on rate of anaphase movement or FAURÉ-FREMIET's on the rate of segmentation in *Ascaris* eggs. The position of the optimum is, of course, correlated with the temperature at which the two organisms live (e. g. 40° C. in the chick, 37° C. in *Ascaris*), and above this temperature the cells often suffer damage.

If R_T and R_{T+10} are the rates of a process at temperatures T° C. and (T + 10)° C. then Q_{10} is given by $\frac{R_{T+10}}{R_T}$. For a chemical reaction in artificial media and at similar temperatures, e. g. the catalytic hydrolysis of an ester by H ions, Q_{10} is usually between 2 and 4. It does not show very rapid changes with the temperature interval considered.

The Q_{10} for a single observed biological process is, however, the resultant of the Q_{10}s of numerous reactions going on in the cell which may affect the process under consideration. We therefore generally find very great fluctuations in Q_{10} according to the temperature considered and indeed, within a range of much less than 10° C. Hence Q_{10} calculated over a temperature interval of 10° C. may give a completely erroneous picture.

As an example we may take BUCCIANTE's calculation of Q_{10}. The times of anaphase at 21° and 31° C. are 24.37 and 4.47 mins respectively. So Q_{10} according to BUCCIANTE is 2.37/4.47 = 5.45. Similarly Q_{10} from 35° C. to 45° C. = 3.05/2.00 = 1.52 (see Table 5). Although we have an optimum curve, this fact is entirely concealed by these Q_{10} values, which never fall below unity.

We can show the variations in terms of Q_{10} only if we lessen the temperature interval over which we calculate. Bucciante's experiments were done at intervals of 1° C.

Q_1 is given by $\frac{R_{T+1}}{R_T}$

and Q_{10} in terms of Q_1 is given by

$$\log Q_{10} = 10 \log Q_1$$

Or to generalise where T and T_o are the two temperatures with rates $R_T + R_{To}$

$$\log Q_{10} = \frac{10}{T - T_o} \log \frac{R_{To}}{R_T}$$

Table 5 gives the Q_{10} calculated by this method compared with BUCCIANTE's.

FAURÈ-FREMIET (1925) has investigated the effect of temperature on the velocity of segmentation of *Ascaris* eggs. BUCCIANTE compares these data with his own on the time of anaphase. FAURÉ-FREMIET's Q_{10} values which are given in Table 7, are apparently very different from those calculated from BUCCIANTE's data. Some of these values are negative. It is difficult to understand what a negative Q_{10} means for any biological process, or how these values were calculated. Q_{10} is a ratio of two velocities and segmentation is an irreversible change, as is the anaphase movement. A negative Q_{10} is therefore impossible. I have recalculated the figures

Table 7. Q_{10} for velocity of segmentation of *Ascaris*.

Temperature range ° C.	Q_{10} FAURÉ-FREMIET	TRUE Q_{10}
0—16	6.25	4.38
16—23	3.93	6.25
23—32	1.84	1.84
32—37	0.00	0.96
37—42	— 4.1	0.05

given by FAURÉ-FREMIET (l. c.). They are given in Table 7, and it will be seen that they show a similar trend to those calculated for time of anaphase. The difference commented on by BUCCIANTE lies simply in the method of calculation. It is apparent that Q_{10} is not a suitable tool for the analysis of such complicated processes as segmentation and anaphase movement.

References.

Baas-Becking, G. M., H. S. v. d. Bakhingzen and H. Hotelling: The physical state of protoplasm. Verh. Akad. Vet. Amst. **25**, 5 (1928). — **Becker, W. A.:** Recent investigations *in vivo* on the division of plant cell. Bot. Rev. **4**, 446 (1938). — **Belar, K.:** Beiträge zur Kausalanalyse der Mitose, II. Untersuchungen an der Spermatocyten von Chorthippus (Stenobothrus) lineatus Panz. Roux' Arch. **118**, 359 (1929a). — Beiträge zur Kausalanalyse der Mitose, III. Untersuchungen an den Staubfadenhaarzellen und Blattmeristemzellen von *Tradescantia virginica*. Z. Zellforsch. **10**, 73 (1929b). — **Bonner, T. and F. Addicott:** Cultivation *in vitro* of excised Pea Roots. Bot. Gaz. **99**, 144 (1937). — **Bucciante, L.:** Ulteriori richerce sulla velocità della mitosi nella cellule coltivate in vitro in funzione della temperature. Arch. exper. Zellforsch. **5**, 1 (1927). — **Buchholz, J. T., L. F. Williams and A. F. Blakeslee:** Pollen tube growth in ten species of Datura. Proc. nat. Acad. Sci. U.S.A. **21**, 651 (1935). — **Chambers, K. and H. C. Sands:** A dissection of the chromosomes of the pollen mother cells of *Tradescantia virginica* L. J. gen. Physiol. **5**, 815 (1923). — **Darlington, C. D.:** The External Mechanics of the Chromosomes. Proc. roy. Soc. Lond. B **121**, 264—319 (1936). — **Darlington, C. D. and P. T. Thomas:** The breakdown of cell division in a *Festuca-Lolium* derivative. Ann. of Bot. **1**, 747 (1937). — **Ephrussi, B.:** Contribution a l'analyse des premiers stades du développement de l'oeuf. Action de la température. Arch. Biol. Paris **44**, 1 (1933). — **Fauré-Fremiet, E.:** La Cinétique du Développement (Paris). — **Fry, H. J.:** Studies on the mitotic figure, V. Biol. Bull. Mar. biol. Labor. Wood's Hole **70**, 89 (1925). — **Fry, H. J. and M. E. Parks:** Studies on the mitotic figure IV. Protoplasma (Berl.) **21**, 473 (1934). — **Gray, J.:** Mechanism of cell-division II. Proc. Cambridge philos. Soc., biol. Sci. **1**, 225 (1925). — *Experimental Cytology*. Cambridge 1931. — **Heilbrunn, L. V.:** The centrifuge method of determining protoplasmic viscosity. J. of exper. Zool. **43**, 313 (1926). — **Hughes, A. F. W.:** J. sci. Instrum. 1939. (In the press.) — **Kato, K.:** Viscosity changes in the cytoplasm during mitosis as indicated by Brownian movement. Mem. Coll. Sci. Kyoto B **8**, 201 (1933). — **La Rue, C. D.:** Longevity of plant cells in tissue cultures. Science (N. Y.) **87**, 240 (1938). — **Lewis, W. H. and M. R. Lewis:** The duration of the various phases of mitosis in the mesenchyme cells of tissue cultures. Anat. Rec. **13**, 359 (1917). — **Ludford, R. J.:** Action of toxic substances upon the division of normal and malignant cells in vitro and in vivo. Arch. exper. Zellforsch. **18**, 411 (1936). — **Manton, I.:** Some new evidence on the physical nature of plant nuclei from intraspecific polyploids. Proc. roy. Soc. Lond. B **118**, 522—554 (1935). — **Pantin, C. F. A.:** Temperature and the viscosity of protoplasm. J. Mar. biol. Assoc. U. Kingd. **13**, 336 (1924a). — On the Physiology of Amoeboid Movement II. British J. exper. Biol. **1**, 519 (1924b). — **Strangeways, T. S. P.:** Observations on the changes seen in living cells during growth and division. Proc. roy. Soc. Lond. B **94**, 137 (1922). — **Vlès, F.:** Sur les variations des ions H au voisinage des oeufs en division. C. r. Acad. Sci. Paris **177**, 643 (1922). — **Wada, B.:** Mikrurgische Untersuchungen lebender Zellen in der Teilung, II. Cytologia **6**, 381 (1935). — **White, P. R.:** Plant Tissue Cultures. Bot. Rev. **2**, 419 (1936). — **Went, F. W. and K. V. Thimann:** Phytohormones. New York 1937.

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THE NUCLEAR CYTOLOGY OF THE GRASS MITE, PEDICULOPSIS GRAMINUM (REUT.), WITH SPECIAL REFERENCE TO KARYOMEROKINESIS.

by

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With 115 figures in the text.

(Eingegangen am 14. Juli 1938.)

Contents.

I. Introduction.

In the last two decades cytologists have brought together overwhelming evidence pointing to an essential uniformity of karyokinesis in both the animal and plant kingdoms. Most prominent among exceptions is the anomalous *Karyomerokinesis* (more briefly, *Merokinesis*) discovered by ENZIO REUTER (1909b) in the grass mite, *Pediculopsis graminum* (REUT.).

In *Merokinesis* — the mitotic *modus operandi* during the early cleavages of the egg of *Pediculopsis* — the karyological phenomena, as described by REUTER, are highly irregular. The chromosomes never condense (i. e. — become chromatic), but remain through all stages of *Merokinesis* as achromatic threads. A nucleus in the ordinary sense is not present for each achromatic chromosome possesses an individual nucleus or karyomere. The karyomere preserves its integrity throughout the entire course of *Merokinesis*, neither fusing with its neighbor karyomeres nor at any point breaking down and liberating its contained achromatic chromosome. Division and separation of the chromosomes

[1] Lydig Fellow of Columbia University, 1937—1938.

takes place within the karyomere and without the mediation of a spindle. Lastly, the karyomere itself possesses a spindle but undergoes a direct division.

These phenomena are of compelling interest, for *Merokinesis* would appear to offer unique opportunity for detailed analysis of what appears to be a highly simplified chromosome. Not only this, but *Merokinesis* occupies a prominent position with regard to Cytologic theory. It appears to be the supreme case of chromosomal continuity throughout a series of successive division cycles. It gave to REUTER the substance for an hypothesis of the phylogeny of metazoan mitosis, and serves to bolster his achromatin hypothesis (1930) of chromosome structure. Lastly, it represents a completely anomalous mitosis for the *Karyomerokinesis* of *Pediculopsis* has found no parallel in any other organism.

The need for a reinvestigation of *Merokinesis* has become especially obvious, for the researches of NORDENSKIÖLD (1909a, 1920, *Ixodes*), SCHRADER (1923, *Tetranychus*), SOKOLOFF (1934, *Gamasus*) and OPPERMANN (1935, *Argas*) have brought to light no evidences of *Merokinesis* in the cleavage divisions *(Tetranychus)* or spermatogenesis of the Acarina investigated. PÄTAU (1936) was especially concerned with the problem of *Merokinesis* and devoted his efforts to a critical study of meiosis and cleavage divisions of the egg of *Pediculoides ventricosus* NEWP. Of all Tarsonemid genera *Pediculoides* is most closely allied to *Pediculopsis*. Indeed, *Pediculopsis graminum* was first described by REUTER (1900) as a member of the genus *Pediculoides*. Despite the close affinity of the two forms, PÄTAU discovered no trace of *Merokinesis* in the cleavage divisions of the egg of *Pediculoides*. Lastly, in a study of the reproductive behavior and parthenogenesis of *Pediculopsis*, COOPER (1937) has figured and noted normal metaphase chromosomes in the early cleavage mitosis of the egg of *Pediculopsis graminum* (REUT.) itself.

It is the purpose of this study, therefore, to present a new analysis of those phenomena that REUTER described and interpreted as *Karyomerokinesis*. In the course of this reinvestigation considerable information concerning the meiosis and fertilization of the egg of *Pediculopsis graminum* REUT. was also assembled. As no detailed study of fertilization has been made — to the writer's knowledge — for any member of the Acarina, and as the peculiar behavior of the chromosomes during maturation throws some light upon the phenomenon described as *Merokinesis*, these data will also be considered in this paper[1].

[1] I wish to express my sincere appreciation to Professor FRANZ SCHRADER, who suggested and directed this research, for many kind efforts on my behalf. Thanks are extended to Professor ENZIO REUTER of Helsingfors, and to Dr. KLAUS PÄTAU of the Kaiser Wilhelm-Institut für Biologie, for favors tendered me. Warm expression of gratitude is made to my wife, RUTH SNYDER COOPER, to ROBERT H. MACKNIGHT of the California Institute of Technology, and to FRANCIS J. RYAN of Columbia University, for aid in the course of this work.

II. Identity of the North American Species of Pediculopsis.

According to EWING's (1934) classification of the higher categories of the Acarina *Pediculopsis graminum* (REUT.), as a member of the Pediculoidinae of the family Tarsonemidae, belongs to the superfamily Tarsonemoidea of the suborder Heterostigmata. This species appears to have been discovered by AMERLING (1861), and was first described taxonomically by REUTER (1900) as *Pediculoides graminum*. Later REUTER (1907) designated the mite as genotype of *Pediculopsis*, a new genus closely allied to *Pediculoides*.

Pediculopsis graminum occurs in intimate association with *Sporotrichium poae* PECK, a fungus parasitic upon grasses and certain varieties of the cultivated carnation. As REUTER (1909a, b, a) has indicated, the European distribution of the mite is an extensive one. It has been recorded from Nebraska and Omaha in the United States by WOLCOTT (1908) under the name *Pediculoides dianthophilus* WOLC., while STEWART and HODGKISS (1908) have recorded *Pediculopsis graminum* (REUT.) from Illinois and New York State.

As the *Merokinesis* of *Pediculopsis* has no parallel in any other organism known, it is of supreme importance that there be no doubt of the taxonomic identity of the material reinvestigated with *Pediculopsis graminum s. str.* It has been pointed out previously (COOPER 1937) that the North American form of *Pediculopsis* morphologically conforms to REUTER's (1900, 1909a) own detailed descriptions of *Pediculopsis graminum*. Furthermore, REUTER (1909c, STEWART and HODGKISS 1908), after examination of specimens from New York State, has specifically acclaimed the American species to be identical with his *Pediculopsis graminum*. However, it has been pointed out (COOPER 1937) that the American form differs from *Pediculopsis graminum s. str.* by possessing a diploid number of six, not four as REUTER (1909b) specified for the Scandinavian apotypes[1]. It was therefore concluded that either the American and Scandinavian forms represent two closely allied but distinct species [*Pediculopsis dianthophilus* WOLC. and *P. graminum* (REUT). respectively] differing in chromosome garniture, or that REUTER erred in his determination of the diploid number of *Pediculopsis graminum s. str.* The latter is the case.

Through the extreme kindness of Professor ENZIO REUTER I have been privileged to study three of his original slides of sectioned apotypes of *Pediculopsis graminum*. There can be no doubt that here as well the diploid number is six. The chromosomes of this apotypic material received from REUTER differ in no evident respects from those of the American form. REUTER (1909c) was correct in stating that the North American species is none other than *Pediculopsis graminum* (REUT). *The material of*

[1] Apotypes (*vice Hypotype*, in use): material upon which supplementary descriptions of species are based. In this case the material is actually *heautotypic*; i. e. — specimens identified with an already described and named species, selected by the nomenclator himself in illustration of his species, but not being recognizable as proterotypes (primary types). The usage is that of SCHUCHERT and BUCKMANN: Ann. Mag. Nat. Hist., VII. s., **16** (1905).

the present investigation is in every way comparable to that of REUTER's *classic studies.*

III. Material and Methods.

The mites used in this study were collected at Flushing, Long Island, New York, during July of 1936. They were taken from *Sporotrichium*-infested heads of grasses, chiefly those of *Agrostis alba* L., *Phleum pratensis* L., and *Poa pratensis* L. Stocks of *Sporotrichium poae* were established in petri dish culture from these parasitized grasses. Wort, potato-dextrose, and prune agars prepared by the Difco Laboratories of Detroit, Michigan, were found to be the most suitable culture media. *Pediculopsis graminum* may be reared in such cultures of *Sporotrichium*, as was accidentally discovered by MOLZ and MORGENTHALER (1912). In such a manner a colony of *Pediculopsis* was maintained in the laboratory from July 1936 to September 1937. Detailed descriptions of the mite and its life cycle will be found in REUTER's works (1900, 1909a, b, c), while the results of a recent investigation of the reproduction of *Pediculopsis* are given by COOPER (1937).

Female mites, containing a sufficient number of eggs in early cleavage stages[1] to warrant fixation, were isolated and cleaned of adherent sporotrich fibers. The ovary and egg-filled oviduct were then dissected from the hysterosoma in a drop of Ringer's solution for invertebrated animals. Following dissection the entire ovary and its attached oviduct were either immediately pipetted into the fixative or, in the case of ALLEN's modification of Bouin[2], the fixative was gently squirted over the dissection while still in the drop of Ringer. In the latter case the dissection was then removed with pipette and rapidly passed through two changes of Allen-Bouin into the final bath of the fixative. In any case, good fixation was obtained only when the eggs were totally removed from the body of the female so that the fixing agent obtained free and rapid access to them.

Fixatives used included Allen-Bouin, Bouin, San Felice, strong and weak Flemming, Benda, Kahle, Carnoy, Henning, Perenyi, Gilson, Petrunkewitsch, and Champy. For nearly every purpose fixation in Allen-Bouin at 40° C gave the most satisfactory results. It is of some interest that Allen-Bouin used at room temperature (23° C) gave decidedly inferior fixation. One part of 2% osmic acid to three parts of Allen-Bouin used at 40° C also gave results of considerable value for some phases of study (i. e., prophase). Bouin and Benda were serviceable, although the latter fixative gave good results only when the eggs were individually pricked with a glass needle immediately on immersion in the fixing bath. Champy was not given a thorough trial, but, like Flemming mixtures, gave inferior results. It is quite possible that special treatment prior to use of these fixatives would enhance their value for this material. The remaining fluids gave atrocious fixation, and cannot be considered suitable for *Pediculopsis* (compare REUTER 1909b). In no case was it found necessary to prolong fixation beyond a few hours.

The fixed material was run up to 85% alcohol and enclosed in sacs formed by alcohol-hardened integuments of eviscerated *Drosophila* larvae. These sacs, occluded with fly viscera, were then run on through the higher-percent alcohols and cleared in xylol, cedar oil, or tetrabromnapthalene, and embedded in paraffin of a melting point of 53°—55° C. Material so embedded cut admirably, and the bulk of the preparations were sectioned at 2—3 micra[3].

[1] Physogastric females appear as somewhat pear-shaped, clear, droplets scattered through the culture of *Sporotrichium*. At this stage the condition of the ovary, the number of eggs and their stages of development, all may be easily ascertained through the transparent wall of the hysterosoma. — [2] ALLENS B 15. — [3] A cooling device for the microtome was found indispensable; see: COOPER and MACKNIGHT: Stain Technol. 12 (1937).

Heidenhain's Iron Haematoxylin and Feulgen were principally used as the nuclear stains, but Aceto-carmine, Centian Violet, Bismark Brown, and Mann's stain were also employed. As counterstain Thiazin Red R was especially useful, while Orange G, Bordeaux Red, and Fast Light Green were also utilized. On the whole, most accurate observation was possible on those preparations which had not been counterstained.

Observations were made entirely with Zeiss optical equipment. 10× and 15× compens oculars in conjunction with 2 mm. Apochromat objectives of N.A. 1.3 and N.A. 1.4, and an Aplanat condensor of N.A. 1.4 served as the working optical systems. The figures were all made by camera lucida, and the magnifications at which they are here reproduced are given in the legends. Many of the figures of the cleavage mitoses illustrate an incomplete nuclear or chromosomal content. Unless indicated as haploid, illustrations rendering less than six chromosomes or karyomeres have been executed from single sections which did not contain entire nuclear complements. The following figures were drawn from more than one section: 19, 20, 21, 31, 32, 35, 36—38, 42—44, 46, 48, 50, 59, 61, 68, 70, 71, 74, 75, 79, 82, 83, 90—92.

IV. Oögonia.

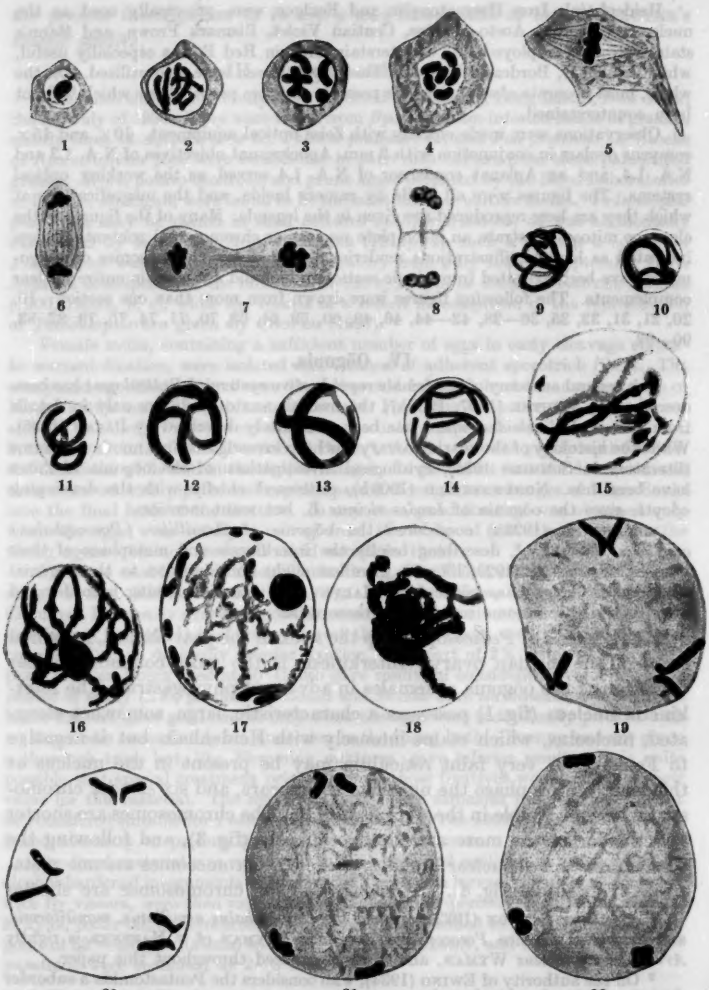
The general anatomy of the female reproductive system of *Pediculopsis* has been described by REUTER (1907, 1909a); the ovarian anatomy differs only in details from that of *Pediculoides*, which has been adequately described by PÄTAU (1936). While the histology of the acarinid ovary has been investigated in a number of forms (literature in SCHMIDT 1935), cytological investigations of the oögonia of mites have been few. NORDENSKIÖLD (1909b), concerned chiefly with the developing oöcyte, gave the oögonia of *Ixodes ricinus* L. but scant mention.

v. HAFFNER (1922a) considered the oögonia of *Armillifer (Porocephalus) armillatus* WYMAN[1, 2], describing briefly the interkinesis and metaphase of their mitoses. SCHRADER (1923) likewise gave but slight consideration to the oögonial divisions of *Tetranychus bimaculatus* HARVEY, his attention having been devoted only to the chromosome numbers in these cells.

The oögonia of *Pediculopsis* cap the apex of the pear-shaped, proximal stem of the median ovary. Interkinesis is the most common nuclear condition of the oögonia of females in advanced physogastria. The interkinetic nucleus (fig. 1) possesses a characteristic, large, somewhat elongated, nucleolus, which stains intensely with Heidenhain but is negative to Feulgen. A very faint reticulum may be present in the nucleus at this time. In prophase the nucleolus disappears, and six slender chromosomes become visible in the nucleus (fig. 2). The chromosomes are shorter and thicker in the more advanced prophases (fig. 3), and following the dissolution of the nuclear boundary, the six chromosomes assume metaphase disposition (fig. 4). At metaphase the chromosomes are shorter

[1] *Armillifer* SAMBON (1922), created for *Porocephalus armillatus, moniliformis,* and *annulatus*. Hence *Porocephalus armillatus* WYMAN of v. HAFFNER is rightly *Armillifer armillatus* WYMAN, and is so designated throughout this paper.

[2] On the authority of EWING (1934), who considers the Pentastomida a suborder of the Acarina, *Armillifer* is treated in this paper as a mite. However, the writer favors HEYMONS' (1926) view that: „Im zoologischen System werden die *Pentastomida* daher zweckmäßig als selbständige Tiergruppe gelten und ihre natürliche Stellung zwischen den *Anneliden* und *Arthropoden* finden, und zwar *am besten* in der Nähe *anderer stummelfüßiger arthropodenähnlicher Tiere, der Tardigraden und Onychophoren.*"



Figs. 1—22. Oögonial mitosis (figs. 1—8) and development of the oöcyte nucleus from leptotene to late diakinesis (figs. 9—22). Oögonia: 1 interkinesis; 2, 3 prophase; 4, 5 metaphase; 6, 7, 8 anaphase-telophase. Oöcyte: 9 leptotene; 10 amphitene; 11, 12 pachytene; 13 early diplotene; 14, 15, 16, 17 diplotene; 18 karyospheroid; 19, 20, 21, 22 diakinesis. 1, 2, 3, 19, 21 Osmicated Allen-Bouin, Heidenhain; 6, 7, 15, 16, 17, 18, 22 Allen-Bouin, Heidenhain; 9, 10, 11, 12, 13, 14 Allen-Bouin, Feulgen; 20 Osmicated Allen-Bouin, Gentian Violet; 4, 5, 8 Navashin, Feulgen. All 3 μ. Magnification for figs. 1—8, 19—22 about 3,225 ×, for figs. 9—18 about 5,200 ×.

and thicker than at any stage of prophase. They are generally arranged in a ring of five encircling one centrally placed, although a ring of six has been observed. Lateral views of metaphase configurations (fig. 5) show the split chromosomes to be equatorially orientated in a biacuminate spindle. The spindle is apparently devoid of asters, centrosomes, and centrioles. In anaphase (fig. 6) the chromosomes evidently separate with their long axes parallel to one another. During their separation the greatest contraction of the oögonial chromosomes is realized. At telophase the spindle appears only as a remnant (figs. 7, 8), and the chromosomes seem to preserve their original metaphasic positions with respect to one another (fig. 7). The interkinetic nuclei arise from the fusion of vesiculated or karyomeric chromosomes (fig. 8).

While the oögonial mitosis of *Pediculopsis* is essentially normal, the period of transient karyomery during telophase is of interest. Both the telophasic karyomery and the parallel displacement of the chromosomes during anaphase reflect peculiarities of the blastomeric mitoses.

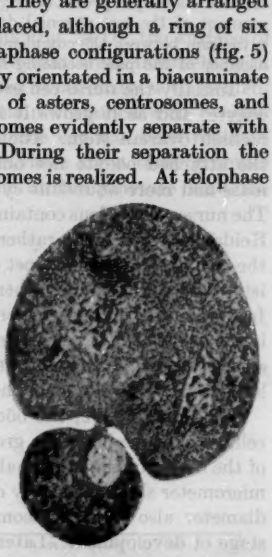


Fig. 23. Nurse cell with attached oöcyte; outlines of figure have been sharpened. Allen-Bouin, Heidenhain. 3 μ. (Photomicrograph by Mr. J. Godrich.)

V. Oögenesis.

1. Cytoplasmic. Inasmuch as Pätau (1936) has given a detailed account of the development of the egg and nurse cell of *Pediculoides*, and, as the course of oögenesis in *Pediculopsis* quite closely follows his description, only a brief discussion will be given here. Pätau has correctly adduced that Reuter (1907) mistook the oöcytes of *Pediculopsis* for the nurse cells and *vice versa*, and for this reason Reuter's conclusions concerning oögenesis and dimegaly of the eggs in this mite are invalid. Nordenskiöld (1909b) has given an account of the oögenesis of *Ixodes ricinus* L. (= *reduvius* Latr.), and v. Haffner (1922a) has briefly dealt with the development of the egg of *Armillifer armillatus* Wyman. Figures of the developing egg and slight references to oögenesis in other acarinids will be found in the literature concerning the histology of mites (see especially, Schmidt 1935).

From late pachytene, each oöcyte of *Pediculopsis* may be observed to be attached by a communicatory stem to a single, much larger, nurse cell (fig. 23). While this state of affairs persists throughout oögenesis in *Pediculopsis* and *Pediculoides*, it appears not to have been discovered in other mites[1]. The oöcyte occupies a peripheral position with respect to the ovarian components, the nurse cell serving as the proximal attachment to the ovary.

As Pätau (1936) has pointed out, and as Knaben (1934) believes he has demonstrated for the moth *Tischeria*, such a plasmatic communication between a nurse

[1] I have observed a similar nurse cell-egg relationship in a unidentified member of the *Tyroglyphidae*.

cell and its oöcyte probably originates through failure of severance of the spindle remnant of the last oögonial division. There would thus be formed a bridge of cytoplasm between the complementary cells of the last gonial division — the inner member of the pair becoming the nurse cell in *Pediculopsis* and *Pediculoides*.

Initially the nurse cell grows at a much higher rate than its attached oöcyte, and as it grows it is moved posteriorly from its origin in the median ovarian stem. The nurse cell in its earliest stage differs from the attached oöcyte not only by its proximal position in the ovarian mass and more abundant cytoplasm, but by its much enlarged nucleus. The nurse cell nucleus contains numerous bodies which stain intensely with Heidenhain but color rather weakly with Feulgen. The compaction of the early nurse cells against one another imposes upon them a characteristic polyhedral shape, whereas the peripheral oöcytes are at this stage free from any pressure other than that of contact with their corresponding nurse cells. Thus the early oöcytes are of the shape of truncated spheroids (e. g., compare PÄTAU's figs. 3 and 5a, *op. cit.*). At a somewhat later stage of development the nucleus of the nurse cell becomes lobulated.

As stated above, the oöcyte grows less rapidly than does the nurse cell, so presumably fully grown nurse cells may possess oöcytes in any of the stages of the latter half of oögenesis. Measurements with an ocular micrometer show that early oöcytes possessing nuclei of the same average diameter also have cytosomes of similar dimensions and of the same stage of development. Later in development of the egg, nuclear growth is slight or even negative but cytoplasmic growth appears unabated. Comparative measurements of the nuclear and cytosomic diameters of each oöcyte studied were made, and gave a means for more accurately seriating the various early developmental trends in both nucleus and cytoplasm of the oöcyte. Somewhat more than one-hundred consecutive measurements have been plotted, and serve to show in summary fashion the trends of the various processes of egg development (fig. 24).

The early growth of the oöcyte consists of an increase in plasma (fig. 24 A→E), as PÄTAU has also found to be the case for *Pediculoides*. A period then ensues during which growth is characterized chiefly by the accumulation of yolk (fig. 24 E→G), after which addition of plasma again appears as the dominant growth process (fig. 24 G→). This progressive accumulation of plasma gives origin to the "*centroplasm*" of REUTER (1909b), and with the increase in amount of centroplasm the yolk assumes a peripheral distribution. A yolk nucleus is not present during oögenesis in *Pediculopsis*, nor did PÄTAU find one in *Pediculoides*.

NORDENSKIÖLD (1909b) failed to observe a yolk nucleus in *Ixodes*, and apparently *Armillifer* is similarly with out such an organelle (v. HAFFNER 1922a). However, yolk nuclei are known to occur in the oögenesis of some mites [i. e., in the Hydrachnid *Diplodontus despiciens* MULL. (SCHMIDT 1935), in *Halarachne otariae* STED. (STEDING 1924), and in *Trombidium* (HENKING 1882[1])], and are

[1] THOMAE [Zool. Jb. Abt. Anat. 47, (1925)] records the occurrence of yolk nuclei in the oögenesis of *Halacarus basteri*.

found quite generally throughout the Arachnida (KOCH 1925, 1926; KRACZKIEWICZ 1931).

At a stage before the appearance of yolk (fig. 24 D), the surface of the egg becomes dotted with small granules that stain intensely with Mann's stain, with Gentian Violet (fig. 25), and occasionally with Heidenhain following Navashin fixation. They remain unstained with Feulgen treatment. A similar granulated surface of the egg has been reported for *Pediculoides* by PÄTAU (1936). Surface views show these granules to interlace in a complicated fashion, while at the juncture with the

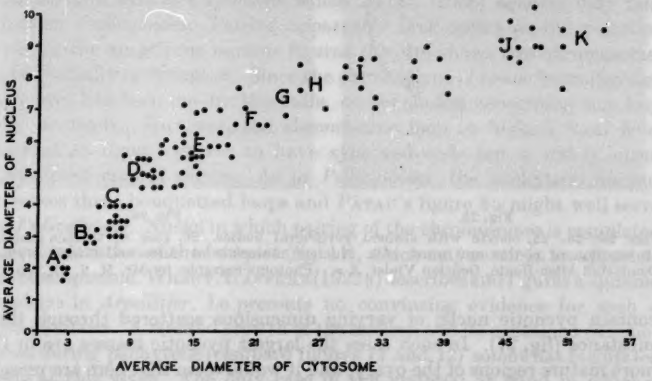


Fig. 24. Plot of measurements of nuclear and cytosomic diameters of more than 100 oöcytes. With the exception of the values of those measurements of the nucleus falling beyond *J*, the individual point represents the average diameter of the nucleus and average diameter of the cytosome for a particular oöcyte. Points beyond *J* represent the average value of the diameter of the cytosome, but the greatest diameter of the nucleus. The values are given in unconverted ocular-micrometer units. The following relations may be shown: (a) Nuclear: $A \rightarrow B$, pachytene; $B \rightarrow I$, diplotene; $F \rightarrow K$, diakinesis; $C \rightarrow I$, diplotene threads stain weakly; $E \rightarrow F$, nucleolus disappears; $I \rightarrow$, cytoplasmic stains color nucleus; $I \rightarrow J$, nucleus may shrink violently on fixation; $J \rightarrow$, nucleus becoming spindle-shaped, volume decreasing, bivalents contracted and may leave periphery of nucleus. (b) Cytoplasmic: $A \rightarrow E$, plasma increase; $E \rightarrow G$, marked deposition of yolk; $G \rightarrow$, growth of centroplasm marked — coincident restriction of yolk to a peripheral ring; $D \rightarrow$, gentiophilic granules present on egg's surface.

nurse cell they form a quite regular ring (fig. 26). However, the granulations are found nowhere upon the surface of the nurse cell itself. Shortly before the oöcyte is liberated from its nurse cell, the staining properties of the egg's surface are no longer peculiar. The granules of the ring at the junction with the nurse cell are the last to disappear. Although no detailed study of this matter was made it seems likely that these granules are concerned with chorion formation. Similar phenomena are so interpreted by NORDENSKIÖLD (1909b) for *Ixodes* and by v. HAFFNER (1922a) for *Armillifer*.

With the completion of oögenesis the egg is no longer attached to a nurse cell and falls free into the ovarian cavity. What the fate of

disjoined nurse cells may be cannot be stated. PĂTAU believes that in *Pediculoides* the nurse cell may degenerate after the liberation of its egg and give rise to a pycnotic nucleus of the type so abundant in older ovaries. The situation appears to be considerably more complex in the case of *Pediculopsis*. Frequently ovaries of *Pediculopsis* are encountered which

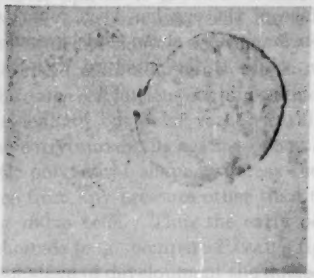


Fig. 25.

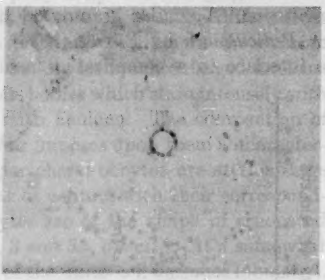


Fig. 26.

Figs. 25—26. 25, oöcyte with stained peripheral bodies. 26, ring of surface granules at junction of oöcyte and nurse cell. Nuclear materials of nurse cell faintly stained. Osmicated Allen-Bouin, Gentian Violet, 3 μ. (Photomicrographs by Mr. M. J. D. WHITE.)

contain pycnotic nuclei of varying dimensions scattered through their substance (fig. 27). In such cases the largest pycnotic masses are in the more mature regions of the ovary, and it would seem that here are present graded stages in the development of the pycnotic nucleus.

Fig. 27. Transverse section through ovary showing pycnotic nuclei in immature cells in the substance of the ovary. The pycnotic nuclei appear as homogeneous dark spots, and are not to be confused with yolk of egg to lower right. Allen-Bouin, Heidenhain, 3 μ. (Photomicrograph by Mr. J. GODRICH.)

2. Karyology. Meiosis of the egg of *Pediculopsis* is a process decipherable only with extreme difficulty. Not only do the various stages of egg development differ in their reactions to staining and fixation, but diplotene nuclei of the same ovary frequently appear to possess mutually contradictory nuclear configurations. In the course of the analysis of early meiotic prophase it was desirable to adopt some means of seriating the nuclear stages quite independent of subjective evaluation. Such a

criterion is afforded by a plot of the comparative measurements of cytosome and nucleus.

It has not been possible to recognize the preleptotene nucleus of *Pediculopsis*, and it is therefore impossible to state what the origin of the leptotene threads may be. At leptotene (fig. 9) the nucleus, which is of the same order of magnitude as the oögonial interphase nucleus, possesses six uniform chromosome loops. The leptotene threads are all polarized at their ends toward a point on the nuclear wall, and give no indication of synaptic synizesis which PÄTAU (1936) believes may take place in *Pediculoides*. Pairing apparently first occurs at the polarized ends for the amphitene nucleus figured (fig. 10) shows two chromosomes to be partially unsynapsed. Since the chromosome of lower focus (lightest in figure) has been cut by the knife, no conclusion concerning this loop may be made. However, the chromosome loop in highest focal level (darkest in figure) proves to have synapsed ends but a widely open, unsynapsed central region. As in *Pediculoides*, the pachytene nucleus possesses three bouquetted loops and PÄTAU's figure 8a might well serve for *Pediculopsis*. Nuclei in which pairing of the chromosomes is completed show a relaxation of the bouquet (fig. 11), which may result in an apparent but false spireme. While v. HAFFNER (1922a) describes and figures a spireme prophase in *Armillifer*, he presents no convincing evidence for such a state of affairs. A marked growth of the nucleus and its contained chromosomes during pachytene (compare figures 11 and 12) somewhat facilitates study at this stage. The only case in the material at hand in which a partial split is visible following the completion of pairing and prior to diplotene is represented in figure 12. While the indication of a split in the pachytene chromosome of highest focal level (darkest in figure) may indeed actually be a region in which synapsis had failed, it also seems possible that here is present the first indication of the so-called diplotene split. If such be the case, the very region which is believed to synapse last during pairing in this chromosome is first to evince the diplotene rift. Early diplotene (fig. 13) shows an obvious medial split running the length of the not-twisted chromosomes.

Clear figures of the diplotene threads following opening out of the bivalents are indeed difficult to obtain because of the extreme minuteness of the figures as well as their tendency to stain irregularly with Heidenhain. Feulgen preparations alone seem useful at this stage and figure 14 — the best of all at hand — shows each of the three bivalents to have two points at which they appear to possess chiasmata. At a still later stage of diplotene — in which the chromosomes are much larger — bivalents possessing two points of apparent chiasmatization are still to be found (fig. 15). Such clear figures are scarce for through the entire course of diplotene the bivalents generally stain very weakly and remain peripherally disposed about the inner surface of the nucleus. For this reason,

only occasionally — in a favorable section containing a surface view of the nucleus — will an entire or sufficiently complete bivalent be found for study. Most of the figures in sections of late diplotene nuclei show only segments or optical sections of the chromosomes (fig. 17), and no account of the structure of the bivalents at this stage is possible. It is, however, probably at this very stage that reduction of chiasmata takes place.

Diffuse nuclear configurations such as illustrated for *Pediculoides* by PÄTAU (1936, figs. 8b, c, e) are frequently to be observed in early oöcyte nuclei of *Pediculopsis*. But prolonged study of nuclei of this size range invariably revealed figures which were amenable to analysis and with few exceptions these showed quite normal diplotene configurations (figs. 13, 14, 15, 16). Because in each range of nuclear size of the early oocyte of *Pediculopsis* there have been found normal diplotene chromosome configurations, it is held that the "diffuse stage" of diplotene for both *Pediculoides* and *Pediculopsis* actually is descriptive only of nuclei fixed and stained in such a manner that interpretation of their structure is rendered difficult or impossible. The course of diplotene and the structure of the bivalent at this stage may well be normal, but demonstration of this is not easy for the chromosome threads are very long, peripherally distributed, and customarily stain so weakly that their differentiation from the nuclear coagulum is poor.

The exceptional diplotene figures in *Pediculopsis* (fig. 18) simulate the karyosphere formations found by BAUER (1933) frequently to be present in the oöcyte nuclei of polytrophic ovaries of insects. While PÄTAU has described the formation of a karyosphere and subsequent differentiation of the bivalents from such a structure in *Pediculoides* (see figs. 8d, f, g in PÄTAU 1936), the karyospheroid of *Pediculopsis* must be viewed with suspicion. It has been pointed out above that the chromosome threads through leptotene, pachytene and early diplotene of *Pediculopsis* are peripherally disposed within the nucleus, and in late diplotene they are in the same manner distributed about the nuclear membrane. In every case observed the diakinetic chromosomes are approximately equally spaced about the nuclear wall (figs. 19—22) and are not clumped to one pole of the nucleus. Furthermore, the massing of the chromosomes into a karyospheroid in *Pediculopsis* cannot represent any single, definite period of diplotene, for karyospheroids may be present at any stage of diplotene following the initial opening of the bivalents. It is therefore concluded that the formation of a karyosphere does not take place normally in the oöcyte of *Pediculopsis*. The karyospheroids of the diplotene nucleus of the oöcytes of this mite are artifacts because the bivalents take their origin and complete their condensation while peripherally disposed about the inner surface of the nucleus, and because there appears to be no constant nuclear and cytosomal sizes at which clumped configurations are

found exclusively. If the formation of the karyospheroid in *Pediculopsis* were not an artifact, it would be expected that the diakinetic chromosomes would first appear clumped together towards one pole of the nucleus as is found to be the case in *Pediculoides* (PÄTAU).

With the increase in length of the chromosome threads during the growth of the oöcyte of *Pediculopsis*, there is a coincident decrease in the staining capacity of the chromosome. Advanced diplotene nuclei contain chromosomes which stain only faintly with Feulgen or Heidenhain. PÄTAU (1936) likewise found the staining quality of the growing oöcyte nucleus of *Pediculoides* to diminish from pachytene until chromosome condensation became pronounced (in karyosphere), and inferred from this a lengthening and loosening of the chromosome thread in diplotene. The problems awakened by such staining phenomena have been discussed in detail by BAUER (1933).

While it is not possible to state the origin of the oöcyte nucleolus in *Pediculopsis*, it forms a very conspicuous structure in the mid-diplotene nucleus (figs. 16, 17, 18) and disappears shortly thereafter (fig. 24, from E → F). The nucleolus is negative to Feulgen, but stains well with Heidenhain, Gentian Violet and Mann's stain. Generally there is but one nucleolus in a nucleus, although oöcytes possessing two nucleoli are occasionally to be observed. PÄTAU (1936) has noted in *Pediculoides* that the nucleolus apparently arises in pachytene at the pole of the nucleus at which the ends of the chromosomes are bouquetted. Such may be the case in *Pediculopsis* for, as a polar view of a mid-diplotene nucleus shows (fig. 16), each of the chromosomes appears to have at least one end terminating at or in the nucleolus. The nucleoli are invariably vacuolated in the larger diplotene nuclei (fig. 17), and at diakinesis nucleoli are no longer observed. Similar vacuolization of the nucleolus has been reported for both *Armillifer* (v. HAFFNER 1922a) and *Ixodes* (NORDENSKIÖLD 1909b) during growth of the oocyte. In *Ixodes* the nucleolus apparently persists to a very advanced stage of oögenesis.

Oöcyte nuclei of *Pediculopsis* at the maximum of their growth contain chromosomes in advanced stages of diakinetic contraction (figs. 19, 20). The bivalents at the earliest stages of diakinesis in which they may be studied lie approximately equally spaced from one another on the internal surface of the nucleus, and this distribution is maintained until prometaphase of the first maturation division (figs. 19, 20, 22, 28). The diakinetic nucleus of the oöcyte is from thirty to more than one-hundred times the volume of the spermatogonial or oögonial nucleus in *Pediculopsis*. Thus the even distribution of the bivalents on the periphery of the nucleus is hardly to be accounted for by DARLINGTON's (1937) repulsion hypothesis unless the interchromosomal repulsions are considered to be of great magnitude (compare DARLINGTON, op. cit., page 103).

It is unfortunate that it was not possible to obtain information concerning the nature of chiasma-reduction during diplotene, for although the bivalents appear to have two chiasmata each in early diplotene they are at diakinesis indisputably devoid of any visible chiasmata that might bind their clearly separated halves. Each chromosome of the bivalent at early diakinesis possesses two arms (fig. 19), one of which is considerably shorter than the other. These differential arms are set at an obtuse angle to one another so that each half bivalent appears as a widely open V, one arm of which is considerably extended. The corresponding differential arms of the chromosomes of a bivalent lie opposite each other and approximately in the same plane. The angulate apex of each chromosome is the point of closest approximation of the halves of the bivalent. The whole constitutes a symmetrical figure which with startling clarity demonstrates that the condensing post-diplotene bivalent of *Pediculopsis* is not held together by chiasmata (figs. 19, 20, 21). That the separated halves of each bivalent are indeed internally divided into chromatids follows from the structure of the leftmost lower bivalent of figure 19. Here the distal terminus of the innermost long arm of the bivalent is viewed from above and may be seen to be clearly divided. In one preparation (fig. 20) two of the bivalents possess a delicate connecting thread between the angulated apices of their separated halves. These threads may indeed be artifacts for they stain weakly and quite dissimilarly to the body of the bivalents. A further consideration of these structures is given below.

Condensation of the diakinetic bivalent of *Pediculopsis* to its metaphase form apparently comes about by a pronounced contraction of the longer differential arm. Intercalated between the differential arms lies a relatively achromatic region (lower right bivalent, fig. 20, which represents a normal structure of the chromosomes of *Pediculopsis* for it is also evident during the mitotic divisions of cleavage. The small size of the chromosomes and the difficulty in obtaining precise staining, however, makes it impossible to demonstrate cytologically the achromatic region in all chromosomes and at all stages in *Pediculopsis*. At advanced diakinesis (fig. 21) the bivalents have the two arms of each paired chromosome of nearly equal length. In the concluding stages of condensation the two arms are much contracted, and of the same length, and give to each of the half-bivalents the appearance of two closely appressed spheroids (fig. 22). The recognition of a biarmed condition of the chromosomes is furthered by the presence of the achromatic constriction. The differentiation between the two moieties of each half-bivalent may be lost as the tetrads attain their metaphase form (fig. 28, 29, 30), although the sharpest figures may still show evidences of the biarmed condition of the paired chromosomes even at late metaphase stages. The chromosomes united in the bivalents of *Pediculopsis*, therefore,

are laterally associated. Detailed consideration of tetrad structure in *Pediculopsis* and notes on other Acarina are given below (pages 97—99).

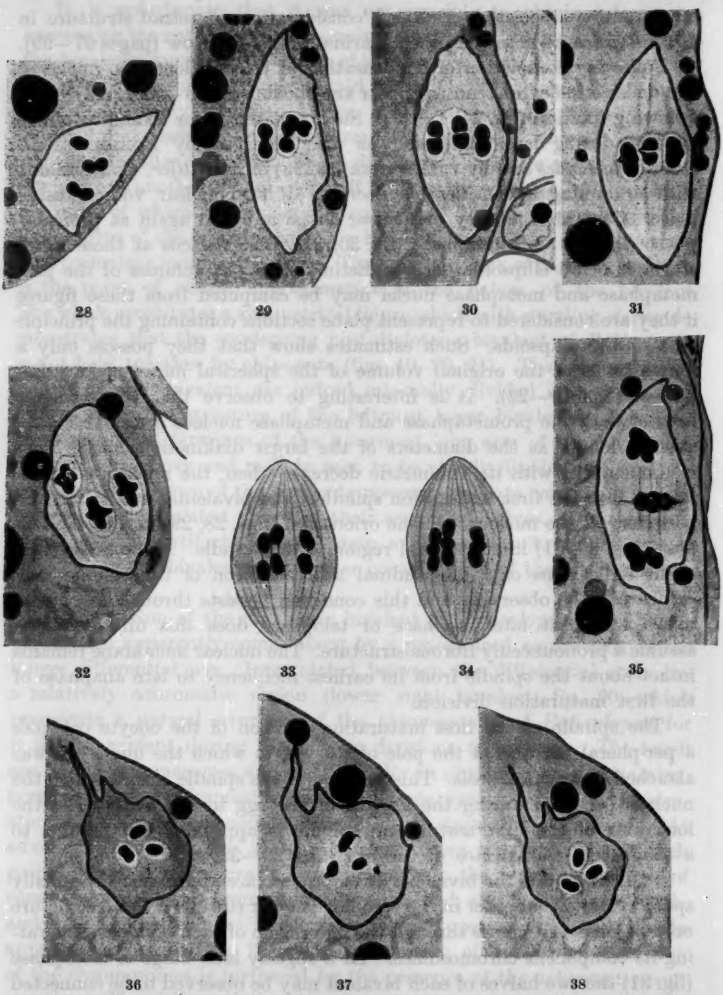
Following completion of condensation of the bivalent, the nuclei of late diakinetic öcytes tend to appear amoeboid in shape — or crumpled — following fixation (fig. 24, I → J). Such apparent poor fixability of the nucleus during late oögenesis was also observed by NORDENSKIÖLD (1909 b) in *Ixodes* and by V. HAFFNER (1922 a) in *Armillifer*. Concomitant with crumpling a considerable decrease of the nuclear volume takes place. General regularity of nuclear shape is found again at prometaphase (fig. 29) and metaphase (fig. 30, 31). The nucleus at these latter stages is of an ellipsoidal shape. Estimates of the volumes of the prometaphase and metaphase nuclei may be computed from these figures if they are considered to represent plane sections containing the principle axis of the ellipsoids. Such estimates show that they possess only a fourth or so of the original volume of the spherical nuclei of late diakinesis (figs. 19—22). It is interesting to observe that the principle axes of both the prometaphase and metaphase nucleus are of the same average length as the diameters of the larger diakinetic nuclei[1].

Concurrent with its volumetric decrease, then, the nucleus is transformed into the first maturation spindle. The bivalents, having left the periphery of the nucleus, become orientated (figs. 28, 29) in a metaphase plate (fig. 30, 31) in the central region of the spindle. At prometaphase slight indications of a longitudinal differentiation of the spindle substance may be observed, and this condition persists through early anaphase. Only at late anaphase or telophase does this differentiation assume a pronouncedly fibrous structure. The nuclear membrane remains intact about the spindle from its earliest incipiency to late anaphase of the first maturation division.

The spindle of the first maturation division of the oöcyte occupies a peripheral position at the pole of the egg to which the nurse cell was attached during oögenesis. This position of the spindle is that which the nucleus occupied during the growth of the egg in the ovary, and the long axis of the first maturation spindle is approximately parallel to a tangent to the surface of the egg (figs. 29—32, 35).

In *Pediculopsis* the bivalents at metaphase are approximately equally spaced from one another in the spindle. A clear rift in which no structure may be observed passes through the mid region of each bivalent, separating its component chromosomes. At a slightly later stage of metaphase (fig. 31) the two halves of each bivalent may be observed to be connected by a pair of delicate threads or arms which stain intensely with Heidenhain but are negative to Feulgen. At later stages of metaphase the

[1] Thus whereas all values to J in figure 24 represent *average* diameters of the oöcytes, values from J → represent *greatest* diameters of the oöcytes. This accounts for the levelling off of the curve.

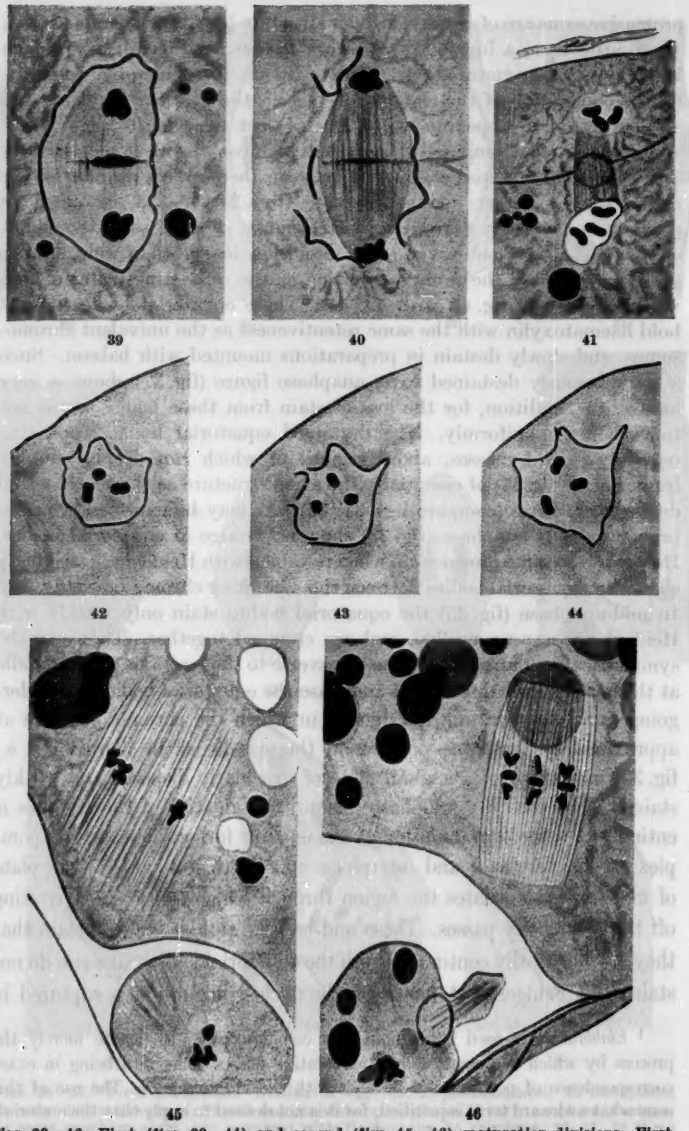


Figs. 28—38. First maturation division of egg. 28, 29 prometaphase; 30 metaphase; 31 early elaborative phase; 32 mid and late elaborative phase; 33 early anaphase, Feulgen; 34 same anaphase as 33, but restained with Heidenhain; 35 anaphase; 36, 37, 38 three transverse levels through anaphase figure — 37 plate of destained equatorial bodies, 36, 38 polar groups of univalents. 28, 30, 31, 35 Allen-Bouin, Heidenhain. 29, 32, 36—38 Osmicated Allen-Bouin, Heidenhain. 33 Navashin, Feulgen; 34 same figure as 33 but restained with Heidenhain. All 3 μ, magnification about 3,225 ×.

protrusive arms are of coarser, heavier structure (fig. 32, bivalents to left). These arms form a bipartite framework between the members of which Feulgen-negative materials accumulate in not inconsiderable amounts. At the completion of the elaborative phase[1], the bivalent may simulate a cross tetrad in appearance (fig. 32, bivalent to right).

In anaphasic disjunction the two halves of each bivalent move widely apart, and the material elaborated between them is left resident in the region of the former metaphase plate (figs. 34, 36—38, 42—44). In preparations strongly stained with Heidenhain (figs. 42—44), the equatorial bodies may be observed to be somewhat longer than wide, and to possess essentially the same orientation as the separating plates of univalents (compare fig. 43 with 42, 44). These equatorial bodies do not hold haematoxylin with the same retentiveness as the univalent chromosomes, and slowly destain in preparations mounted with balsam. Such a spontaneously destained early anaphase figure (fig. 37) shows a very interesting condition, for the loss of stain from these bodies seems not to take place uniformly. The destained equatorial bodies appear as regularly-shaped masses, about regions of which run deeply staining frameworks (fig. 37) of essentially the same structure as those elaborated during metaphase (compare figs. 31, 32). As may be expected, Feulgen preparations of anaphase (fig. 33) show no vestige of equatorial bodies. However, the same preparation when restained with Heidenhain strikingly shows the equatorial bodies between the separating chromosomes (fig. 34). In mid-anaphase (fig. 35) the equatorial bodies stain only weakly with Heidenhain, appear swollen, and are clumped together. They may be symmetrically doubled in a plane transverse to the long axis of the spindle at this stage. Probably at mid-anaphase the equatorial bodies are undergoing rapid degeneration, for figures in which the chromosomes are at approximately the same position in the spindle as in figure 35 (i. e., fig. 39) may show only a small plate of irregularly disposed and weakly stained materials. By telophase (fig. 40) the identity of these bodies is entirely lost, and a granulation at the equator forms the mid-body complex of the telophase and interphase spindle (figs. 40, 41). This plate of mid-bodies delineates the region through which the plane separating off the polar body passes. These mid-bodies, while it is not certain that they are genetically continuous with the equatorial bodies, likewise do not stain with Feulgen. At telophase the nuclear membrane is ruptured in

[1] *Elaboration* is used throughout this communication to denote merely the process by which the equatorial or elaborative bodies come into being in exact correspondence of position and number with the chromosomes. The use of this somewhat awkward term is justified, for it is not desired to imply that the materials of the equatorial body are *eliminated*, *diminuted*, or *secreted* by the chromosomes. Whereas it is not unlikely that we are here dealing with an elimination of materials from the chromosomes (*sensu* BAUER 1933), demonstration of such is lacking at this time.



Figs. 39—46. First (figs. 39—44) and second (figs. 45—46) maturation divisions. First division: 39 late anaphase; 40 telophase; 41 interphase, polar body cut off; 42, 43, 44 three transverse levels through anaphase figure — 43 plate of equatorial bodies; 42, 44 polar groups of univalents. Second division: 45 elaborative metaphase, polar body to lower right; 46 early anaphase reconstructed from two sections, polar body lower left. 39, 40 Allen-Bouin, Heidenhain. 41, 46 Osmicated Allen-Bouin, Heidenhain. 42—44 Flemming, Heidenhain. 45 San Felice, Heidenhain. All 3 μ, magnification about 3,225 ×.

several places (fig. 40). At interphase (fig. 41) no vestige of the original nuclear membrane may be demonstrated. The structure of the spindle becomes markedly fibrous concomitant with dissolution of the nuclear membrane.

At metaphase the half-bivalents may show indications of the medial achromatic region (i. e., fig., 31), but such cases are not frequent. In one nucleus figured (fig. 32) the splits separating the chromatids of some of the half-bivalents are quite evident. During anaphase it would appear that one end of each univalent is in advance of the other (fig. 35, 39). Polar views of the univalents at anaphase may show clearly the achromatic constriction (especially figs. 42, 44) as well as the longitudinal split (fig. 42, univalent to the extreme left). It appears that the univalents, following the elaboration of the equatorial bodies, have a general loosening of their structure (relaxation of coiling?) for at telophase (fig. 40) and interphase (fig. 41) they appear as widely open, short V's at the apices of which the achromatic constriction resides.

Throughout the entire course of the first maturation division — from late diakinesis to interphase (figs. 21—22; 28—41) — the chromosomal elements are enveloped by a non-staining area which appears as a sharply delimited halo. Such a halo is likewise found about the equatorial bodies following their separation from the chromosomes (figs. 37, 43). At interphase (fig. 41) the chromosomes of both the polar body- and egg-groups remain condensed, and do not form either karyomeres or nuclei. The chromosomes of both the egg and polar body are surrounded by a non-staining halo comparable to that which envelops the individual chromosomes during the course of the first maturation division.

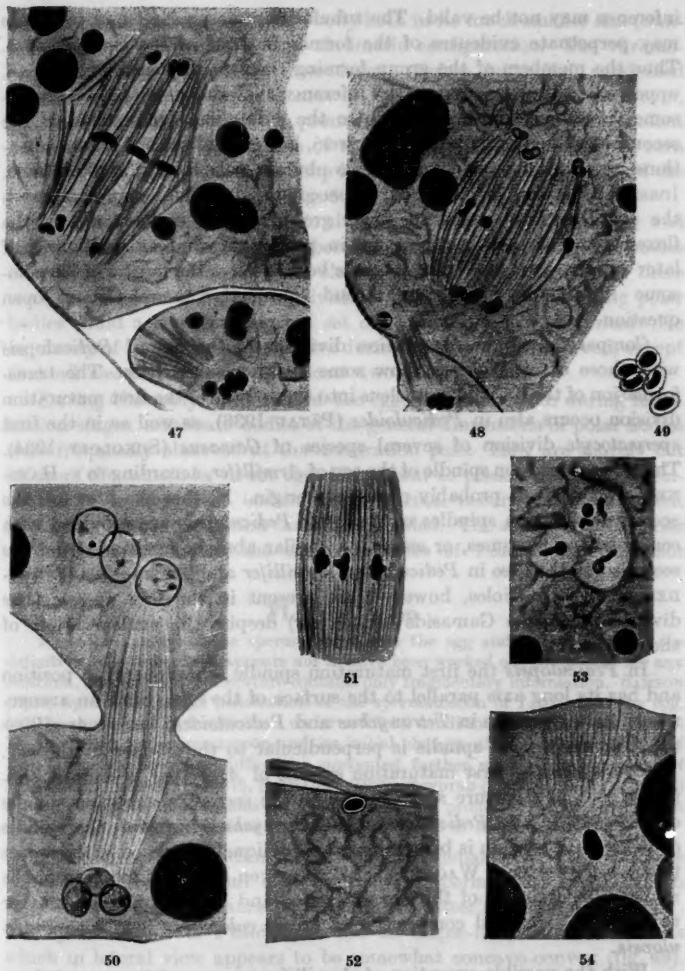
In *Pediculopsis* the strongly-fibrous, cask-shaped spindle of the second maturation division is nearly perpendicular to the surface of the egg. The three univalents of *Pediculopsis* are evenly distributed on the equatorial plate at early metaphase, and show a clear separation between their component chromosomes (fig. 45, two leftmost univalents). Both components of a univalent are shaped like a widely-open V, and juxtaposed so that their apices are most closely approximated. In this division, as in the first maturation division, Feulgen-negative equatorial bodies are elaborated. Here again they appear between the separated chromosomes (fig. 45, right) prior to anaphase, and with the completion of their formation the univalents may simulate cross-tetrads in appearance (fig. 51). As in the case of the first maturation division, no resemblances of the chromosomes to cross-tetrads can be noted if the preparations are restained with Feulgen. Non-staining halos may frequently be observed to envelop the chromosomes during metaphase of the second maturation division also.

The early anaphase figures (fig. 46) are unusual in that the ends of the V-shaped chromosomes preceed in the movement while the apices lag. The equatorial bodies remain in the plane of the former metaphase

plate, perpetuating the metaphase position of each univalent. From polar view the configuration of early anaphase (fig. 53) is unique, for the equatorial bodies of the second maturation division at this stage appear as symmetrical rings between pairs of separating chromosomes. Like the equatorial bodies of the first maturation division, those of the second maturation division stain intensely with Heidenhain but are completely negative to Feulgen. They also may appear to be split during the course of the second division. Unlike those of the first division, however, the equatorial bodies of the second maturation division may persist to telophase (figs. 47, 48). As the equatorial bodies are not to be found in interkinetic stages (fig. 50), their disintegration must take place during telophase.

The telophase chromosomes (fig. 47) lose their staining capacity and become negative to Feulgen as well as to Heidenhain. The chromosomes then appear as closed tubes or elongated vesicles delimited by definite membranes (fig. 48). The tubular chromosome undergoes a great increase in volume, and during this volumetric increase it loses its tubular shape and becomes spherical. Thus an individual nucleus or karyomere is formed from each chromosome. These karyomeres retain their individuality, not fusing to form a single nucleus. Figures of completed telophase have narrower but more elongated spindles than those of early telophase, and they are devoid of equatorial bodies. Whether or not mid-bodies are present at this time seems to depend largely on the staining process; the more heavily the figure is stained the more likely are mid-bodies to be present (see FRY 1937). At each pole of the late telophase spindle rest there is a group of three karyomeres (fig. 50). These karyomeres possess only faint indications of internal structure. The only constant internal structure appears to be a single, Feulgen-negative, nucleolus in each karyomere. The group of karyomeres remaining within the egg forms the female pronucleus. Having attained their full spherical dimensions, the karyomeres constituting the female pronucleus leave the spindle as a group and undergo the movements and changes described in the section on fertilization (Section VI).

Whereas the actual achromatic constrictions of the chromosomes of *Pediculopsis* have not been observed during the course of the second maturation division, their positions during metaphase and anaphase of this division are sharply marked by the pronounced medial or nearly medial flexure of each chromosome (figs. 45, 48, 53). At telophase (fig. 47) the chromosomes still evince a marked division into two arms, and their configuration suggests that the ends of the chromosomes lead throughout the entire poleward movement. However, inasmuch as fixation has produced marked longitudinal splits in the spindle of this figure and by this token is not good, and as at least one of the chromosomes (fig. 47, lower left chromosome) has been displaced in sectioning, the



Figs. 47—54. Second maturation division (figs. 47—48, 50—51, 53) and fertilization (figs. 49, 52, 54). Second division: 47 early telophase, polar body to lower right; 48 telophase with chromosomes vacuolate, polar body to lower right; 50 late telophase, female pronucleus (above) and second polar body; 51 elaborative metaphase; 53 early anaphase in polar view with four chromosomes and all three equatorial bodies visible. Fertilization: 49 cluster of spermatozoa from spermatheca of female; 52 spermatozoön just under chorion; 54 spermatozoön penetrated to peripheral ring of yolk. 47, 49, 52, 53 Allen-Bouin, Heidenhain. 50, 51 Osmicated Allen-Bouin, Heidenhain. 48 Flemming, Heidenhain. 54 Navashin, Feulgen. All 3 μ, magnification about 3,225 ×.

inference may not be valid. The tubular chromosomes of mid-telophase may perpetuate evidences of the former flexures of the chromosomes. Thus the members of the group forming the female pronucleus (fig. 48, upper) all appear to support the inference that the ends of the chromosomes preceeded the mid-regions to the pole. The components of the second polar body group (fig. 48, lower), however, show but scant indications of possessing two arms and no obvious polarization of their ends. Inasmuch as the members of the second polar body complement in the anaphase figures (fig. 46, lower group) appear to have one of the flexed arms of each chromosome in precession, and as no figures of later anaphase are available, it seems best to leave the nature of chromosome movement during the second maturation anaphase an open question.

Comparison of the maturation divisions of the egg of *Pediculopsis* with those of other mites show some striking similarities. The transformation of the diakinetic nucleus into the spindle of the first maturation division occurs also in *Pediculoides* (PÄTAU 1936), as well as in the first *spermatocyte* division of several species of *Gamasus* (SOKOLOFF 1934). The first maturation spindle of the egg of *Armillifer*, according to V. HAFFNER (1922a, b), is probably of nuclear origin. Neither the first nor the second maturation spindles of the egg of *Pediculopsis* are provided with centrioles, centrosomes, or asters. A similar absence of these structures seems to be the case in *Pediculoides*, *Armillifer* and *Tetranychus* (SCHRADER 1923). Centrioles, however, are present in the first *spermatocyte* division of certain Gamasids (SOKOLOFF) despite the nuclear origin of the spindle.

In *Pediculopsis* the first maturation spindle is peripheral in position and has its long axis parallel to the surface of the egg. Such an arangement is also the case in *Tetranychus* and *Pediculoides*, but in *Armillifer* the first maturation spindle is perpendicular to the surface of the egg. The barrel-shaped first maturation spindle of *Armillifer*, therefore, conforms both in structure and position to the second maturation spindle of *Pediculoides* and *Pediculopsis*. In *Tetranychus*, however, the spindle of the second division is biacuminate and obliquely disposed with respect to the egg's surface. WAGNER (1894) has given but brief mention of the maturation divisions of the egg of *Ixodes*, and his data are too meager to allow any detailed comparison of *Ixodes calcaratus* BIR. with *Pediculopsis*.

With the possible exception of *Armillifer* (see page 94), none of these mites appear to give any indications of the elaborative phenomena so conspicuous in the maturation divisions of *Pediculopsis*.

3. Polar Bodies. In *Pediculopsis*, unlike *Pediculoides* (PÄTAU 1936) and *Tetranychus* (SCHRADER 1923), the first polar body generally does not divide during the second maturation division (figs. 45—48). It differs

markedly from the second polar body both in its much smaller size (compare figs. 45—48 with 50) and chromatic configuration. The chromosomes of the first polar body do not form a nucleus, but remain condensed (figures cited). The chromosomes of the second polar body, however, simulate the behavior of the pronuclear group and form independent karyomeres. These karyomeres of the second polar body differ in turn from those of the pronuclear group in that the volumetric increase of the latter karyomeres is greater (fig. 50).

The polar bodies may divide during the cleavages of the egg, and their divisions are frequently aberrant. As many as six polar bodies have been observed at the fifth cleavage of the egg, and four of these were in advanced anaphase of division. In none of these dividing polar bodies could a complete haploid set of chromosomes be discerned. No regularity of number of polar bodies during cleavage was observed, except that all cleaving eggs have at least two polar bodies.

During the early cleavages of the egg, or indeed even during fertilization stages, anucleate bodies of the general dimensions of polar bodies were frequently observed at the maturation pole. They are sharply cut off from the remainder of the egg, and appear to possess delimiting membranes. Whether these bodies are of normal occurrence or represent pernicious effects of fixation cannot be decided. The subsequent histories of the polar bodies and the anucleate blebs of *Pediculopsis* were not investigated.

VI. Fertilization.

The actual entry of the spermatozoön into the egg and its evolution to the definitive male pronucleus appears not to have been worked out completely for any Acarinid, and the extant literature contains only fragmentary information. Samson (1909) figures the initial penetration of the spermatozoön of *Ixodes* into the egg, but the case is one of fertilization under artificial conditions. v. Haffner (1922b) has described the spermatozoön and its initial changes after entry to the egg of *Armillifer*, but technical difficulties prevented further study of the process of fertilization. Reuter (1909b), Schrader (1923), Steding (1924), and Pätau (1936) have all recorded observations on the union of the pronuclei in the fertilization of mite eggs, but give no data on the earliest stages in the process.

The spermatozoa within the inseminated female of *Pediculopsis* are clumped in the canaliculi of a complicated spermatheca interposed in the oviduct a short distance posterior to the latter's origin on the ovary. The spermatozoön of *Pediculopsis* has an ellipse-like, flattened nucleus which in lateral view appears to be somewhat concavo-convex (fig. 49). The nucleus stains very intensely with Feulgen and other nuclear stains. It is surrounded by a highly refringent, non-staining envelope which gives an ellipsoid form to the spermatozoön.

These spermatozoa of *Pediculopsis* are apparently of an immotile type, and are evidently of more simplified organization than those of the investigated Mesostigmate Acarina, viz., — Parasitoidea (Gilson 1888; Michael 1892; Steding

1924; Sokoloff 1934) and Ixodoidea (Leydig 1855; Heller 1858; Pagenstecher 1861; Christophers 1906; Bonnet 1906, 1907; Nordenskiöld 1909a, 1920; Samson 1909; Casteel 1917; Warren 1933; Tuzet and Millot 1937), and seem to correspond more closely to the type found by Michael (1884) in the Cryptostigmate *Damaeus*. If Sambon (1922) is correct that the Linguatulids are truly Acarina, then it is very interesting that the highly specialized parasite *Armillifer* possesses the apparantly rather primitive flagellated spermatozoön described by v. Haffner (1922b). Such a flagellated spermatozoön is wholly unknown elsewhere among the Acarina[1]. Although Pagenstecher (1860) has described the spermatozoön of *Trombidium* as possessing a flagellate tail, his observation has not been confirmed by Henking (1882), Thor (1904), or Warren (1933). Thor investigated the spermatozoa of six species, representing four families (Hydrachnidae, 3 spp.; Erythraeidae, Trombidiidae, and Anystidae, 1 sp. each), of Prostigmates, but none of these possess flagellated spermatozoa. All of these Prostigmates appear to have spermatozoa with short and thick, worm-like tails[2], and these spermatozoa seem to constitute a wholly unique type among the investigated Acarina[3].

As stated above in the section concerning the karyology of maturation, eggs released from their nurse cells into the ovarian cavity are found to be in the metaphase of the first maturation division. Similarly eggs in the pre-spermathecal oviduct as well as in the passage of the spermatheca are also in first metaphase. As eggs which have passed the spermatheca into the so-called uterine portion of the oviduct are never to be found in the metaphase stage of the first maturation division, but always at some later stage, it seems clear that entrance of the spermatozoön to the egg takes place either during the first maturation metaphase or shortly thereafter. In agreement with this conclusion are the earliest cases of sperm penetration in the present material (table 1). v. Haffner (1922b)

Table 1. Summary of the available data on the fertilization of the egg of *Pediculopsis graminum* (Reut.)

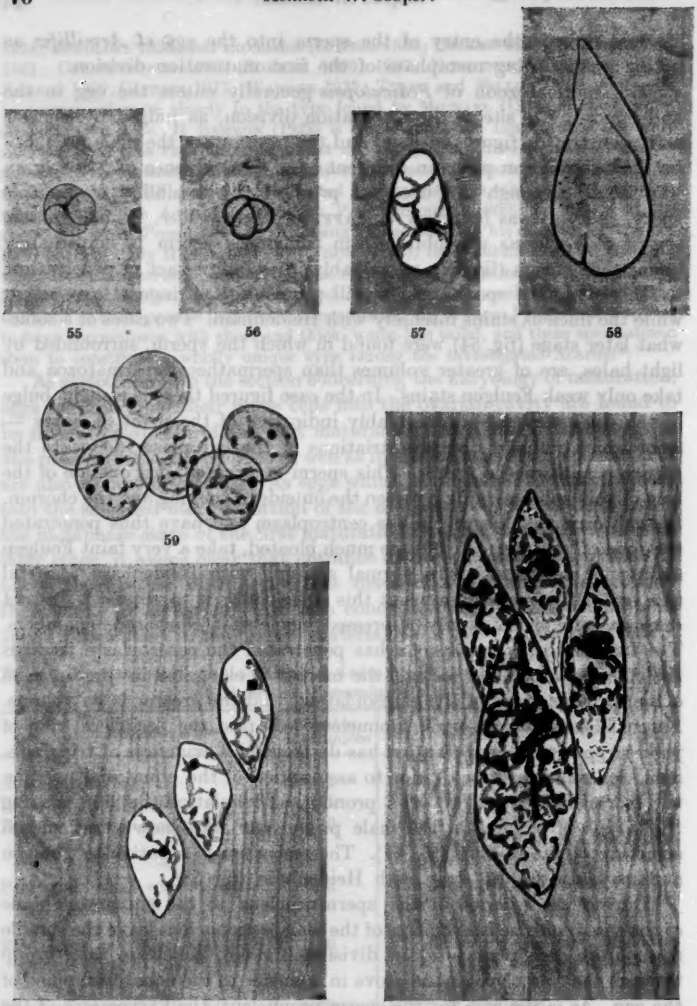
Sperm nucleus	Number of examples	Female or egg nucleus
At surface of egg	1	First maturation metaphase
Entering egg (fig. 52)	1	First maturation metaphase
Between chorion and yolk ring (fig. 54)	2	One in first maturation metaphase; one in second maturation metaphase
Past yolk ring, centroplasmic, swelling (figs. 55, 56)	2	One in anaphase of second maturation; one in telophase of second maturation
Elongating and increasing in volume (figs. 57, 58)	10	Female pronucleus on copulation path, karyomeres elongating and swelling
Union of pronuclei (fig. 61)	10	Union of pronuclei

[1] Indeed, Heymons (1926) cites the structure of the spermatozoön of the Pentastomid as a character separating the tongue worms from the mites and other Arachnoids. — [2] Claparède (1868, *Atax* and *Tetranychus*), Henking (1882) and Warren (1933) appear to have been dealing with spermatids rather than mature spermatozoa. See also Michael (1896) on *Bdella* (Eupodoidea). — [3] See, however, Michael's (1892) figure of the spermatozoön of the Parasitid *Sejus*.

likewise records the entry of the sperm into the egg of *Armillifer* as taking place during metaphase of the first maturation division.

The spermatozoön of *Pediculopsis* generally enters the egg in the half opposite the site of the maturation division, as judged by the few early penetration figures at hand and the positions of the male pronuclei on their copulation paths in different eggs. No evidence of polyspermy was found, although this does not preclude the possibility of multiple sperm entry as was found by V. HAFFNER in *Armillifer*. In one case an egg of *Pediculopsis* was obtained in which the sperm is immediately under the chorion (fig. 52), presumably in the initial act of penetration. At this stage the spermatozoön still possesses a refringent investment, while the nucleus stains intensely with Heidenhain. Two cases of a somewhat later stage (fig. 54) were found in which the sperm, surrounded by light halos, are of greater volumes than spermathecal spermatozoa and take only weak Feulgen stains. In the case figured there is a slight bulge of the egg's surface — presumably indicative of the point of entry — from which radially directed striations of the cytoplasm delineate the entrance path of the sperm. This sperm is situated just outside of the ring of yolk spheres, lying between the outside of the ring and the chorion. Spermatozoa that lie within the centroplasm and have thus penetrated the zone of yolk (figs. 55, 56) are much bloated, take a very faint Feulgen stain, and are negative to normal staining with Heidenhain. Careful analysis of the sperm nucleus at this stage shows it to possess a distinct division into three closely appressed, somewhat elongated, chambers. The further the spermatozoön has penetrated the centroplasm towards the equatorial plane of an egg, the more it is elongated in the direction of the principle axis of the ellipsoidal egg and the greater is its volume. Figure 57 is that of a male pronucleus well past the peripheral ring of yolk. Apparently poor fixation has destroyed all evidences of tripartiteness, for at a stage just prior to assumption of the equatorial position the sperm nucleus still shows a pronounced lobulation (fig. 58). During the stages of elongation the male pronucleus may show a reticulated structure of the nucleus (fig. 57). The reticulum is negative to Feulgen and may also fail to stain with Heidenhain (fig. 58).

During the passage of the sperm nucleus to the equatorial plane of the egg, the three karyomeres of the female pronucleus leave the spindle rest of the second maturation division (fig. 50), penetrate as a group through the ring of yolk, and move in a cluster to the equatorial plane of the egg. As is the case for the sperm pronucleus, the karyomeres of the female pronucleus elongate and increase in volume during their copulatory movements. Eggs in which the male and female pronuclei have closely approached one another have their centroplasm markedly striate along the principle axis of the egg (fig. 61), as described by REUTER (1909 b).



Figs. 55—61. Fertilization (figs. 55—58, 61) and cleavage (figs. 59, 60) of egg. Cleavage division indicated by Roman numeral following classification of figure. Fertilization: 55 trilobed male pronucleus past yolk ring and just in centroplasm; 56 male pronucleus as in 55, but in lateral aspect; 57 male pronucleus on copulation path; 58 male pronucleus shortly before reaching equator; 61 gonomeric prophase of first cleavage, three smaller karyomeres compose the female pronucleus. Cleavage: 59 interkinesis I, from two sections; 60 early prophase II, three karyomeres only. 55 Navashin, Feulgen. 56 Allen-Bouin, Heidenhain. 57, 58, 59, 60, 61 Osmicated Allen-Bouin, Heidenhain. All 3 μ, magnification about 3,225 $\times$.

The male pronucleus and the maternal karyomeres form a group of four elongated vesicles at the equator of the egg, thus completing the process of fertilization. While the male pronucleus even at this stage may evince its tripartite nature, it does not dissociate to form three independent karyomeres in the material examined. Here at the equator of the egg, prophase ensues (fig. 61), and as the karyomeres do not fuse with one another a consequent gonomery of the cleavage nucleus results. Inasmuch as the succeeding phenomena are essentially the same as those occurring in the mitoses of the blastomeres of early cleavage, a description of the first cleavage mitosis will be deferred. Suffice it to say that each of the karyomeres of maternal origin elaborates a single chromosome, while the trivalent male pronucleus gives rise to three chromosomes. At telophase of the first cleavage division each of the six chromosomes gives rise to a single vesicle or karyomere.

It is to be noted especially that in *Pediculopsis* the cleavage nucleus consists of four elongated karyomeres only, for the male pronucleus does not resolve itself into independent vesicles. Thus it is not at all remarkable that REUTER mistook the chromosome number of *Pediculopsis graminum* as four, because the number of karyomeres (which REUTER held to be enduring throughout the entire mitosis) of the cleavage nucleus of the fertilized egg was his ultimate criterion of the chromosome number.

It is quite impossible to ascertain what the condition of the male and female pronuclei of *Halarachne* may be from the data given by STEDING (1924). However, SCHRADER (1923) describes the female pronucleus of *Tetranychus* as consisting of three karyomeres while the male pronucleus is figured as a single large, irregular vesicle which may be divided internally. Such is essentially the condition in *Pediculopsis*. But it is interesting to note that PĀTAU (1936) describes and figures the paternal pronucleus of *Pediculoides* as composed of three independent, spherical karyomeres. Like REUTER (1909b) PĀTAU believes that the sperm resolves itself into karyomeres within the egg. Such may well be the case even if the male pronucleus of *Pediculoides* is originally a single vesicle, for HUGHES-SCHRADER (1931) has given an indisputable example of a nucleus (spermatocyte of *Llaveia*) dividing into independent karyomeres during prophase.

Nevertheless, PĀTAU's figure 9i may receive another interpretation. It will be noted that the supposed two groups of karyomeres in this figure possess quite identical patterns. As the illustration is a reconstruction from two sections, it is quite possible that it represents two transverse levels through one and the same elongated maternal pronucleus of an haploid egg which may not have been properly superimposed. Likewise WAGNER (1894, fig. 17) figures a cluster of separate bodies (karyomeres?) as the male pronucleus in the egg of *Ixodes*, but it seems quite likely that he has misinterpreted his sections. It is here suggested (in the absence of further information, for WAGNER's German resumé gives no adequate description of his materials) that the supposed male pronucleus of WAGNER's figure is actually the female pronucleus and that the chromosome group at the periphery of the egg is a polar body complement.

VII. Karyomerokinesis.

1. Resumé of REUTER's *Findings.* It was in the early cleavage divisions of the egg of *Pediculopsis graminum* (REUT.) that ENZIO REUTER (1909b)

discovered the extraordinary nuclear phenomena which led him to announce discovery of an entirely new type of mitosis. Because REUTER's classic monograph is not generally accessible, a resumé of his interpretation of the cleavage mitoses of *Pediculopsis* is desirable.

The resting stage of mitosis in the early cleavage divisions of the egg of *Pediculopsis* is, according to REUTER, characterized by the occurrence of a karyomere or individual nucleus for each of the four chromosomes of a set (fig. 62a). Within each such vesicle there resides a chromatic blob to which is appended an achromatic tail. This achromatic thread is held by REUTER to be the permanent component of the chromosome in each vesicle, whereas the chromatic granule is a transient structure for it is not found during all stages of a mitosis. The karyomere does not fuse with adjoining karyomeres in early cleavage divisions, but in fact remains independent and intact throughout the entire course of a mitotic cycle.

With the onset of prophase, the vesicle elongates and at this stage the chromosome within it consists of a double achromatic thread possessing a prominent terminal chromatic granule. The distal end of the split chromosome likewise has a small, deeply-staining body binding the two achromatic threads (fig. 62b). In later prophase (fig. 62c), the distal ends of the achromatic threads are freed, apparently by division of the distal granule into two terminal bodies. In late prophase the karyomere becomes markedly attenuate at each end of its long axis, while the chromosome dyad (*vide* REUTER) dissociates into its component members following division of the proximal chromatic granule (fig. 62d, e). At this stage the achromatic sister chromosomes may have both their ends terminated by chromatic blobs, although this may not always be the case. The long axes of the karyomeres of a set are parallel to one another, and the cytoplasm adjacent to the karyomeres becomes coarsely striated in the direction of the principle axes of the elongated vesicles (fig. 62d, e). The attenuated karyomeres assume a metaphase arrangement and about each elongated vesicle forms a sheaf-shaped spindle (fig. 62f). The karyomere, now at its stage of greatest elongation, is constricted or scalloped medially. Within the vesicle the sister achromatic chromosomes, now *J*-shaped and devoid of all terminal chromatic granules, pass to opposite halves of the karyomere.

Frequently, but not invariably, a chromatic body may be wrapped about the medial constriction of the karyomere (fig. 62f, g). These chromatic bodies (fig. 62h) are held by REUTER to be entirely independent of the chromosomes for they are found to be inconstant in number. Furthermore, they are never included within the karyomere and they degenerate following separation of the half-karyomeres to the poles of the spindles. Inasmuch as these bodies simulate normal chromosomes both in form and staining reactions, REUTER gave them the non-committal term "chromosomoids". Occasionally the mitotic figure is complicated by still another element. This additional complication consists of parallel rows of granules (fig. 62g) on either side of the chromosomoid. These elements REUTER interprets as the degenerating ends of spindle fibers.

Having one achromatic thread or chromosome segregated in each moiety, the karyomere constricts completely in half. The halves of each karyomere, much as separating sister chromosomes during a normal mitotic anaphase, then pass to opposite poles of the spindle (fig. 62j). Through the equator of the spindle passes the plane of cleavage of the blastomere. During this anaphase each achromatic thread regains a terminal chromatic granule (fig. 62i, j). The somewhat elongated karyomeres become somewhat shorter and rounder (fig. 62k) and the mitotic cycle is complete with the onset of the resting stage. A rain of chromatic bodies appears in the spindle (fig. 62k) during telophase of this extremely aberrant mitosis, and these represent the degeneration products of the chromosomoids. To this mitosis

of chromosomal vesicles or karyomeres, REUTER quite appropriately gave the name *Karyomerokinesis*, or, more briefly, *Merokinesis*.

2. Cleavage. As described by REUTER (1909a), the first cleavage of the egg of *Pediculopsis* is total, in a plane perpendicular to the long

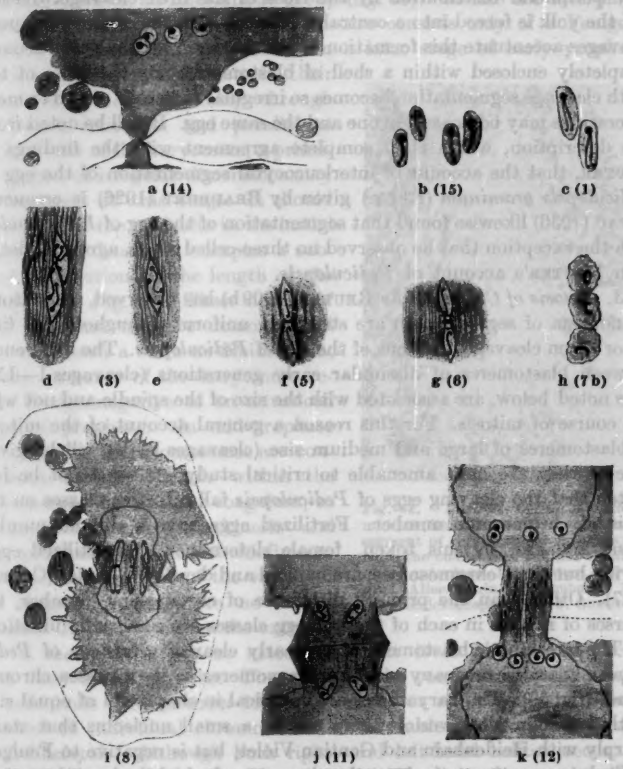


Fig. 62 a—k. *Karyomerokinesis*, from REUTER (1909 b). Whereas the figures are presented here in a different order from REUTER's own, they retain their original numbering. Figs. 3, 11, 12 and 14 not entire. See text for description.

axis of the ovum, and cuts the egg into equal blastomeres. Each of these blastomeres halves in a plane perpendicular to that of the first cleavage, and generally the second cleavage planes are at right angles to each other. REUTER has noted that one of the first two blastomeres may cleave before the other resulting in a three-celled stage. While eggs of *Pediculopsis* possessing but three blastomeres have been observed during the course

of the present investigation, such a condition may not represent the customary progress of segmentation. Be that as it may, cleavage again becomes regular following the culmination of the formation of the delayed cleavage plane of the second division. The blastomeres attain an essentially peripheral distribution by the close of the fifth cleavage division and the yolk is forced into a central position within the egg. Subsequent cleavages accentuate this formation of a blastoderm for the yolk becomes completely enclosed within a shell of blastomeres. By the close of the ninth cleavage segmentation becomes so irregular that cells of two or more generations may be present in one and the same egg. It will be noted from this description, which is in complete agreement with the findings of REUTER, that the account of interleucocytal segmentation of the egg of *Pediculopsis graminum* (REUT.) given by BRADBURY (1926) is erroneus. PĂTAU (1936) likewise found that segmentation of the egg of *Pediculoides*, with the exception that he observed no three-celled stage, agrees in detail with REUTER's account of *Pediculopsis*.

3. Mitosis of Cleavage. As REUTER (1909 b) has observed, the mitotic phenomena of segmentation are strikingly uniform throughout the first six or seven cleavage divisions of the egg of *Pediculopsis*. The differences between blastomeres of dissimilar early generations (cleavages I—IX), as is noted below, are associated with the size of the spindle and not with the course of mitosis. For this reason a general account of the mitosis of blastomeres of large and medium size (cleavages I—V) will be given here as they are most amenable to critical study. It must not be forgotten that the cleaving eggs of *Pediculopsis* fall into two classes on the basis of chromosome number. Fertilized eggs have a diploid number of six and are, by this token, female determined. Unfertilized eggs, having but three chromosomes, are haploid and develop as males (COOPER 1937). Other than the primary difference of chromosome number, the courses of mitosis in each of the two egg classes are essentially identical.

The interkinetic blastomere of the early cleavage divisions of *Pediculopsis* contains as many nuclei or karyomeres as it possesses chromosomes (fig. 59). The karyomeres are spherical in shape and of equal size. Within each nuclear vesicle there resides a small nucleolus that stains sharply with Heidenhain and Gentian Violet, but is negative to Feulgen. A Feulgen-negative reticulum, the character of which is dependent upon the fixative used, is present. While the nucleolus may occasionally be situated at the focal point of a number of threads of the reticulum, it is not constantly provided with any achromatic appendages in the material studied.

In prophase the karyomeres remain in a group and elongate in such a manner that their long axes are parallel to one another. This elongation takes place in the direction of the future metaphase spindle, and generally at right angles to the long axis of the spindle rest of the preceeding mitosis.

The cytoplasm about the elongating vesicles is free of yolk, and becomes coarsely striated parallel to the principal axes of the attenuated karyomeres (figs. 60, 63—68).

Vesicles in earliest prophase (fig. 60) differ in shape and orientation from members of an interkinetic group (fig. 59), but like the interphase karyomeres their visible internal structure consists only of a Feulgen-negative nucleolus and reticulum. In a later stage of early prophase (fig. 65) the karyomeres attain their fullest elongation. Running through the coarse reticulum and parallel to the long axis of the karyomere, a principal thread of irregular dimensions may be discerned at this stage. It should be noted that the nucleolus in the initial stages of condensation occupies a subterminal position on the length of the chromosome (fig. 65). These details of early prophase may be demonstrated only with such a stain as Heidenhain, for the contents of the karyomere remain Feulgennegative to an advanced stage of prophase. In mid-prophase (figs. 63, 64) the chromosomes are conspicuous, gyrate, threads within the karyomeres; the reticular coagulum, so conspicuous during interkinesis and early prophase, is scant and chiefly confined to the periphery of the vesicle at this stage. Nucleoli are no longer present. In mid-prophase the extremities of the vesicles are so attenuated that it is difficult to decide what their limits may be. The gyres of the condensing chromosomes are relic coils (DARLINGTON 1935). As many as seven gyres have been observed in individual chromosomes of favorable mid-prophase figures of *Pediculopsis*. Although both right- and left-hand helices occur, no reversal in the direction of coiling of an individual chromosome has been found. It should be remarked that a karyomeric complement may contain both right-and left-coiled chromosomes. Concomitant with shortening of the chromosomes the number of relic coils is reduced (compare figs. 64, 66, 68). Thus the degree of relic coiling gives an accurate means for ascertaining the relative stages of prophase in a particular cleavage division. While in general all of the chromosomes of a particular karyomeric complement possess roughly the same number of relic coils at each stage of prophase, instances have been noted in which one or two chromosomes

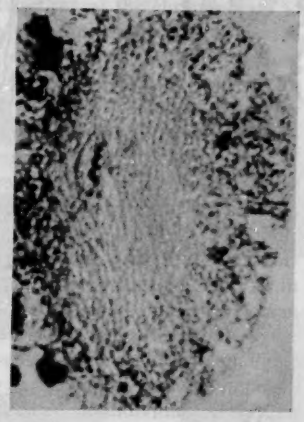
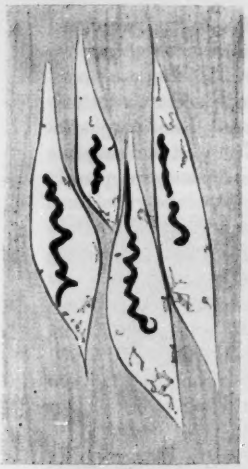


Fig. 63. Portion of an elongated mid-prophase karyomere containing a segment of a relic coiled chromosome. Note the coarse longitudinal striation of the cytoplasm. IV cleavage. Osmicated Allen-Bouin, Heidenhain. 3 μ. (Photomicrograph by Mr. J. GODRICH.)



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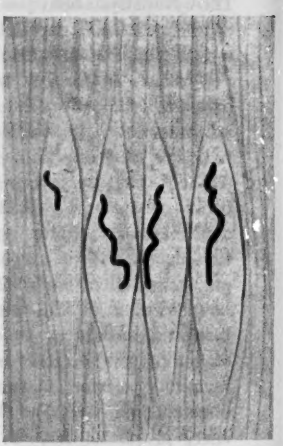

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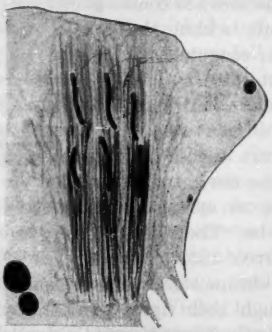

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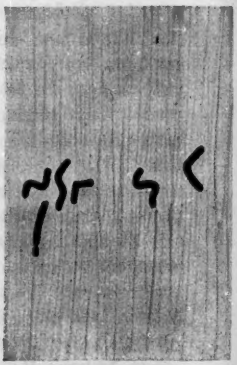

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Figs. 64—70. Cleavage prophase. Cleavage division indicated by Roman numeral following classification of figure. 64 mid-prophase IV; 65 early prophase VI; 66 mid-prophase II; 67 late prophase V, transverse section of karyomeres; 68 late prophase V; 69 early prometaphase VI; 70 late prometaphase IV. 64, 67, 68 Allen-Bouin, Heidenhain. 65 Osmicated Allen-Bouin, Heidenhain. 66, 70 Allen-Bouin, Feulgen. 69 Benda, Heidenhain. All 3 μ, magnification about 3,225 ×.

were in relatively advanced stages of uncoiling as compared with the remainder of the set. In late prophase the chromosomes are pronouncedly shorter and in general retain but a few relic coils. It is at this advanced

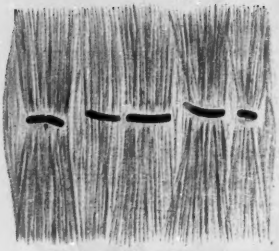
stage of uncoiling that the Feulgen stain and Aceto-carmine first give positive reactions during prophase (fig. 66). The chromosomes now remain positive to Feulgen and Aceto-carmine until telophasic vesiculation.

Just prior to the disappearance of the outline of the karyomeres, the prophase chromosomes appear as elongated threads with little more than faint suggestions of previous relic coiling (fig. 68). Transverse sections of karyomeric groups at such stages reveal the chromosomes to one side of their elongated vesicles (fig. 67). Preceeding the formation of the metaphase plate the karyomeres appear to undergo dissolution. The chromosomes then lie free in the field of fibrillated cytoplasm. In such a prometaphase stage the long axis of the individual chromosome is frequently parallel to that of the forming spindle. Completely symmetrical figures of such prometaphases present a very striking appearance (fig. 69). In late prometaphase the chromosomes, shorter and thicker than at any period of prophase, appear to bend sharply as they come to lie on the equatorial plate (fig. 70).

The well spaced chromosomes form a metaphase plate in the equatorial plane of the cylindrical or barrel-shaped spindle (figs. 71, 72). As in the case of the biacuminate spindles of the oögonial mitosis and first maturation division, the cleavage spindle appears to be devoid of asters, centrosomes and centrioles. Quite frequently the metaphase spindle of early cleavage seems to be formed by the aggregation of smaller spindles, for each chromosome may appear to possess an individual sheaf-like spindle (fig. 71).

At no stage of prophase or prometaphase do the chromosomes possess a visible longitudinal split (figs. 64—70). At earliest metaphase they are likewise unsplit (fig. 71) and thus form an equatorial plate stage (Doncaster 1924). However, in later metaphase the chromosomes exhibit a pronounced longitudinal split (fig. 72). At this stage they appear to have increased in volume, although the figures illustrated here do not allow comparison of volumes. Following the appearance of the longitudinal split the two halves of each chromosome undergo parallel displacement and a stage occurs during which equatorial bodies are elaborated.

Inasmuch as figures which appear to be intermediate (fig. 82) between the split stage of metaphase and that stage at which equatorial bodies are entirely formed are quite infrequent, either the process of elaboration is a rapid one or the material of the equatorial body generally accumulates simultaneously along the whole length of the separating chromosomes. As is the case in the maturation divisions, the equatorial material stains intensely with Heidenhain but is negative to Feulgen. With the culmination of the elaborative stage the sister chromosomes appear as slender threads lying on opposite sides of an elongate, chromosome-like body



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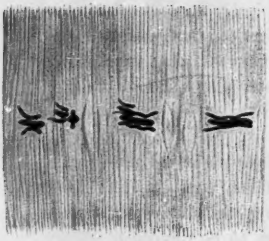

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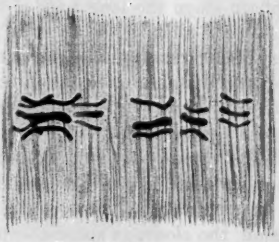

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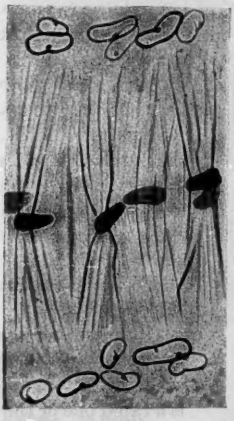

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Figs. 71—79. Cleavage metaphase (figs. 71—73), anaphase (figs. 74—76), and telophase (figs. 77—79). Cleavage division indicated by Roman numeral following classification of figure. 71 early metaphase V; 72 mid-metaphase VII, split visible in chromosomes; 73 complement of late metaphase chromosomes V, elaboration completed; 74 early anaphase IV; 75 anaphase V; 76 anaphase III; 77 early telophase group of haploid egg II, above — one polar group of chromosomes, below — corresponding equatorial bodies; 78 telophase V, initial vacuolization; 79 mid-telophase III, note nucleolar patches in vesicles. 71, 75, 79 Osmicated Allen-Bouin, Heidenhain. 73, 74, 76, 77, 78 Allen-Bouin, Heidenhain. 72 Acetocarmine. All but 72 at 3 μ, 72 smear. Magnification about 3,225 ×.

(fig. 73). Indeed the chromosome threads may even appear to be embedded in opposite surfaces of the equatorial body.

In the case of *Pediculopsis* Aceto-carmine and Feulgen give identical staining reactions at every stage of mitosis. HEITZ (1933) has already noted the general tendency of Aceto-carmine to give a nucleal reaction. The chromosomes of prophase, prometaphase and the equatorial plate stage in *Pediculopsis* all appear to be longitudinally unsplit with Feulgen and Aceto-carmine. But these stains do show a metaphase stage in which the chromosomes possess an obvious longitudinal split (fig. 72), as well as a phase of limited parallel displacement of the chromosomes (fig. 83).

Fig. 80.

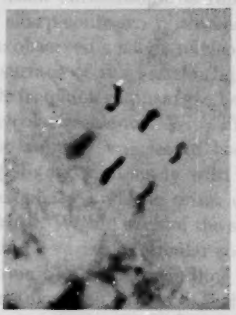


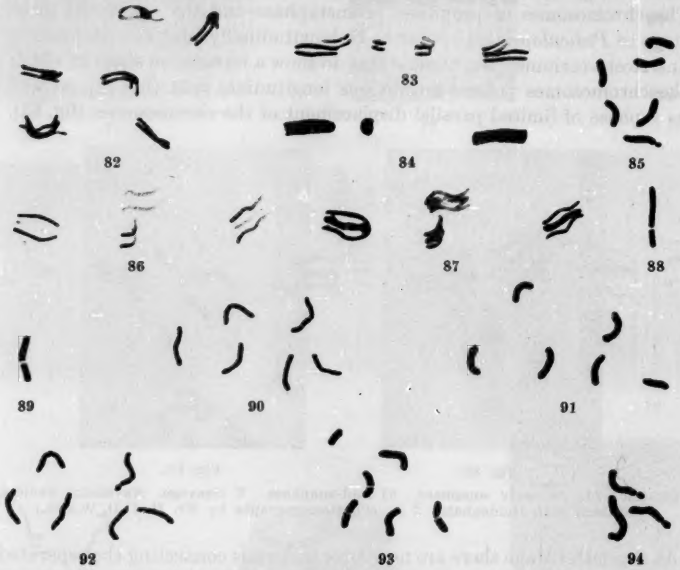
Fig. 81.

Figs. 80—81. 80 early anaphase. 81 mid-anaphase. V cleavage. Navashin, Feulgen restained with Heidenhain. 3 μ. (Photomicrographs by Mr. M. J. D. WHITE.)

At this latter stage there are no visible materials connecting the separated chromatids of each chromosome. The same Feulgen preparation was restained with Heidenhain and the result is very striking (compare figs. 83 and 84). Only faint indications of the separated threads of each chromosome, which stained so sharply with Feulgen, are now visible. The threads lie either on the suface of, or embedded in, elaboration masses which stain intensely with Heidenhain. Early anaphase figures likewise exhibit no stained equatorial bodies between the separating chromosomes in either Feulgen (fig. 86) or Aceto-carmine preparations. Unstained rods, demonstrable only in anaphase and early telophase figures, are the sole visible vestiges of the equatorial bodies. However, Feulgen preparations of anaphase which are restained with Heidenhain show prominent equatorial bodies between the separating sister chromosomes (compare figs. 86 and 87).

In anaphase the chromatids separate from the equatorial body. In their movement to opposite poles sister chromosomes maintain an essentially parallel displacement to one another and to their equatorial body.

Minor ripples may appear along the length of the chromosomes during anaphase. The major flexures of a chromosome, it may be noted, frequently appear mirror-imaged by the major flexures of its sister chromosome. Furthermore, during anaphase the chromosomes become longer. Thus, even in early anaphase, they are frequently longer than their



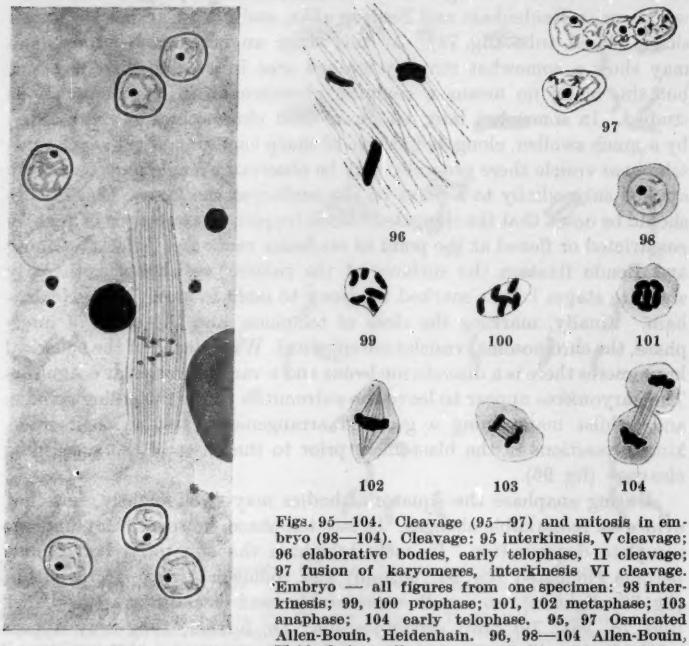
Figs. 82—94. Mitotic chromosomes. Cleavage division indicated by Roman numeral following classification of figure. 82 mid-elaborative (?) metaphase III; 83 elaborative metaphase III, Feulgen; 84 three chromosomes of high focus of fig. 83, but restained with Heidenhain; 85 metaphase plate VI; 86 early anaphase II, Feulgen, equatorial bodies appear as faint lines between separating members of leftmost and rightmost chromosomes; 87 same figure as 86, but restained with Heidenhain; 88 late prophase chromosome showing achromatic constriction VI; 89 prometaphase chromosome showing achromatic constriction VI; 90, 91, 92 early telophase V, corresponding polar groups of chromosomes (90, 92) and equatorial bodies (91); 93 metaphase plate VII, medial achromatic regions demonstrated in three chromosomes; 94 haploid metaphase plate VIII, each chromosome with trabant. 82, 85, 94 Allen-Bouin, Heidenhain. 83, 86, 88, 89, 93 Navashin, Feulgen. 84, 87, 90—92 Navashin, Feulgen restained with Heidenhain. All 3 μ, magnification about 3,225 ×.

corresponding equatorial body. All of these features of anaphase are well illustrated by figures 74—76, 80, 81. At the close of anaphase the chromosomes, still parallel to their respective equatorial bodies and transverse to the spindle axis, are situated slightly beyond the fibrous extremities of the spindle. The original metaphase pattern is preserved by the order of each telophase group of chromosomes as well as by the equatorial bodies (figs. 77, 90—92).

With the culmination of the anaphase movement of the chromosomes to the poles of the spindle, telophasic changes set in which transform the thread-like chromosomes into spherical vesicles. The individual chromosome of early telophase appears to lose its minor irregularities or ripples and seems to undergo a diametric increase of girth. Concurrent with these changes the chromosome stains progressively more weakly with Heidenhain and Feulgen alike, and in appearance suggests a short, closed tube (fig. 78). At this stage an occasional chromosome may show a somewhat strongly stained area in a submedian position, but this is by no means a common phenomenon in the preparations studied. In somewhat later telophase each chromosome is represented by a much swollen, elongated vesicle of sharp contour. Within each mid-telophase vesicle there generally may be observed a single nucleolar body applied submedially to a point on the surface of the vesicle (fig. 79). It should be noted that the elongated vesicle frequently appears to be slightly constricted or flexed at the point of nucleolar residence. With Flemming and Benda fixation the surfaces of the tubular vesicles of both early and late stages have a marked tendency to stain intensely with Heidenhain. Finally, marking the close of telophase and the onset of interphase, the chromosomal vesicles are spherical. Within each of the spherical karyomeres there is a discrete nucleolus and a variable reticular coagulum. The karyomeres appear to leave the extremities of the dwindling spindle, and, whilst maintaining a grouped arrangement, assume their interkinetic positions in the blastomere prior to the onset of the succeeding cleavage (fig. 95).

During anaphase the equatorial bodies may swell slightly, but they evince no remarkable changes. In late telophase, however, they undergo complete degeneration. In early telophase the equatorial bodies may become elongated (fig. 96, bottom) and somewhat irregular in outline (fig. 78, lightest), or swell considerably and remain sausage-shaped (figs. 78, left; 79) until decomposition. Or further, they may appear markedly shrunken (fig. 96, upper left). Finally, in a small number of cases the equatorial body during telophase appears as a symmetrically doubled structure, simulating closely a chromosome of mitotic metaphase (figs. 77, upper two, 79; fig. 96, bottom and upper right). By onset of interkinesis, with the dwindling of the spindle to a mere remnant, the equatorial bodies appear as little more than chromophobic patches in the vicinity of the equator (fig. 95). Inasmuch as the structure of the equatorial body at telophase varies not only with the fixative employed but also in the eggs of one preparation, no insight as to its organization in the living egg can be gained from the fixed material at hand. But few conclusions may be made concerning the equatorial bodies; they are: 1. The equatorial body is elaborated between the separated chromatids of late mitotic metaphase or early anaphase; 2. during anaphase the

equatorial body appears as a uniform, rod-like element, and the aggregate of equatorial bodies of a mitotic figure preserve the pattern of the metaphase plate; 3. the equatorial bodies degenerate during telophase; and, lastly, 4. inasmuch as interkinetic figures prepared by various fixatives so frequently show symmetrically divided degeneration figures of the



Figs. 95—104. Cleavage (95—97) and mitosis in embryo (98—104). Cleavage: 95 interkinesis, V cleavage; 96 elaborative bodies, early telophase, II cleavage; 97 fusion of karyomeres, interkinesis VI cleavage. Embryo — all figures from one specimen: 98 interkinesis; 99, 100 prophase; 101, 102 metaphase; 103 anaphase; 104 early telophase. 95, 97 Osmicated Allen-Bouin, Heidenhain. 96, 98—104 Allen-Bouin, Heidenhain. All 3 μ, magnification about 3,225 ×.

equatorial bodies, the equatorial body may normally be longitudinally split just prior to or during its degeneration.

The spindle, like the equatorial bodies and the reticulum of the interphase karyomeres, varies greatly with the fixative employed and even with the same fixing fluid. For this reason no detailed account of the structure of the spindle can be given here. However, three points of importance are to be noted: 1. The spindle, following fixation with osmicated Allen-Bouin may appear as an aggregate of individual spindles (fig. 71), each of which is built about one chromosome; 2. metaphase, anaphase, and telophase spindles, following fixation with Carnoy, Perenyi, Brucker, Bouin, Kahle, Allen-Bouin and Flemming, frequently are

incised by extensive longitudinal rifts or splits (figs. 78, 79); these rifts, probably to be attributed to fixation, tend to focalize either on the metaphase chromosome or — in later stages — on the equatorial body, although the clefts in the spindle are not always related to these equatorially situated elements; 3. throughout the course of mitosis the spindle of early cleavage is probably a regular cylinder or somewhat barrel-shaped for frequently its structure is such at metaphase following Aceto-carmine and Navashin, and at anaphase and telophase following Navashin; in these cases fixation results in a finely fibrous structure and the spindle is devoid of pronounced splits, bends of fibers, and individualized bundles (fig. 72).

While extensive alterations may be effected in such a regular body by brutal fixation, it is unlikely that highly differentiated, bowed structures, if present in the living spindle, would compositely be resolved by fixation into a regular, uniformly-constructed body. At interphase the spindle degenerates; during its degeneration it becomes increasingly more narrow and somewhat elongated.

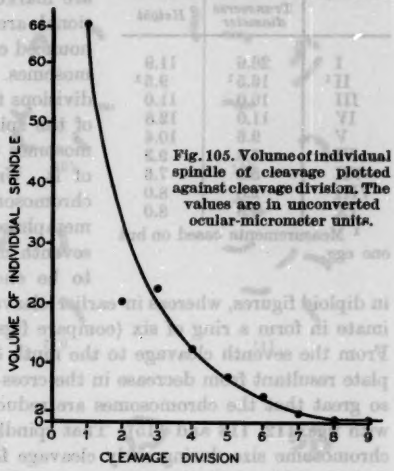


Fig. 105. Volume of individual spindle of cleavage plotted against cleavage division. The values are in unconverted ocular-micrometer units.

The volume of the spindle of the first cleavage division is somewhat more than two-hundred fold that of the average spindle of the tenth cleavage. A plot of the approximate volume of the individual spindle at each of the first nine cleavage divisions of *Pediculopsis* shows the trend of decrease in volume of the individual spindle during cleavage (fig. 105). Measurements of transverse diameter and height of each spindle were made with an ocular micrometer. Although only preparations in which the plane of section was parallel to the longitudinal axis of the spindle were utilized, the measurements are at best rough approximations for they are averaged from specimens prepared by various fixatives. In calculating the volume of the spindle of each of the first nine cleavage divisions, the spindle was regarded to approximate a regular cylinder in form. At the tenth and succeeding cleavages, it should be noted, the spindle is of an entirely different form. The spindles in all mitoses of the embryo from the ninth and following cleavages are biacuminate,

i. e., — spindle-shaped. The data, from which the estimates of spindle volume for figure 105 were made, are given in table 2.

Table 2. Dimensions of mitotic spindle during cleavage; measurements in ocular micrometer units.

Cleavage	Dimensions of Spindle (Average)	
	Transverse diameter	*Height*
I	26.6	11.9
II[1]	16.5[1]	9.5[1]
III	16.0	11.0
IV	11.0	12.8
V	9.6	10.4
VI	7.8	9.3
VII	5.0	7.6
VIII	2.8	8.0
IX	2.8	8.0

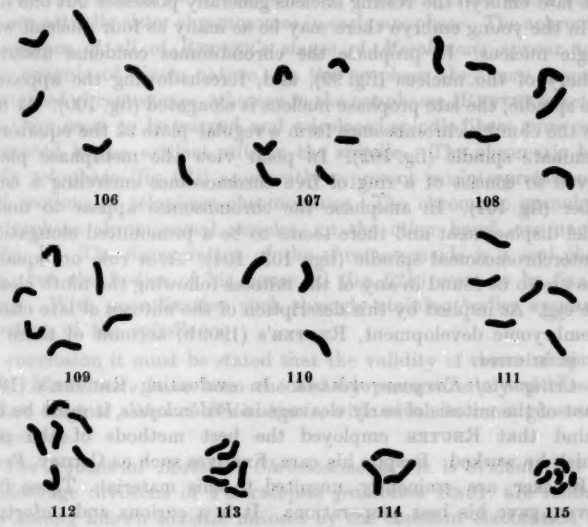
[1] Measurements based on but one egg.

It is evident from table 2 that during the first six cleavage divisions the chief decrease is one of diameter, for the length of the spindle remains fairly constant. Following the sixth cleavage, however, both diametric and longitudinal decreases are marked. The decrease in cross-sectional area of the spindle has a pronounced effect upon the size of the chromosomes. During the first six cleavage divisions the area of a transverse section of the spindle is so great that the chromosomes occupy only a modest fraction of it. Until the seventh cleavage the chromosomes are uncrowded upon the metaphase plate (figs. 106—111). At the seventh cleavage one chromosome tends to be encircled by the remaining five in diploid figures, whereas in earlier cleavages the chromosomes approximate in form a ring of six (compare figs. 106, 107, and 109 with 112). From the seventh cleavage to the ninth the crowding of the metaphase plate resultant from decrease in the cross-sectional area of the spindle is so great that the chromosomes are reduced in length (compare fig. 106 with figs. 112, 113 and 115). That spindle area in *Pediculopsis* controls chromosome size during early cleavage follows from the comparison of diploid and haploid plates at corresponding stages. The areas of haploid and diploid metaphase plates are essentially equal at both the fifth and eighth cleavages (compare figs. 109, 110, 113 and 114). At the fifth cleavage the chromosomes of the haploid and diploid plates are essentially of equal length, and are as long as the chromosomes of the antecedent cleavages. However, if haploid and diploid metaphase plates of the eighth cleavage are compared, it will be seen that the crowded chromosomes of the diploid plate are but approximately seventy-seven percent of their former length whereas those of the uncrowded haploid plate are of undiminished length (compare figs. 113 and 114). These figures of the metaphase plates of the eighth cleavage division are strictly comparable for they are of one and the same preparation.

PĂTAU (1936) has likewise pointed out that in *Pediculoides* the decrease in spindle volume during early cleavage is effected chiefly at the expense of breadth rather than length of the spindle. Furthermore, PĂTAU has shown that a decrease in chromosome length and in area of metaphase plate go hand in hand. If a comparison of PĂTAU's

figures 13e and 19b be made, it will be seen that in *Pediculoides*, like *Pediculopsis*, the haploid plate of the eighth cleavage blastomere is of approximately the same area as the diploid metaphase plate but the chromosomes of the haploid plate are considerably longer than those of the diploid.

While fusions of two karyomeres have been observed in figures of interkineses following the first and fourth cleavages, complete karyomery



Figs. 106—115. Metaphase plates of eggs in successive cleavage divisions. Cleavage division indicated by Roman numeral. 106—II; 107—III; 108—IV; 109—V; 110—V, haploid; 111—VI; 112—VII; 113—VIII; 114—VIII, haploid; 115—IX. All 3 μ, magnification about 2,850 ×. See text.

at interkinesis is the rule for the first five cleavage divisions. At the close of the sixth and succeeding divisions, however, there is an increasingly pronounced tendency for adjacent karyomeres to run together (fig. 97). The chromosomal content of a compound karyomere resulting from such fusions may be read from its nucleolar content. During initial karyomeric fusion (fig. 97, upper), the nucleolus of each karyomere remains distinct. With fusion of adjacent chromosomal vesicles complete the nucleoli may fuse to form a single body (fig. 97, lower). In the latter case the compound nucleolus appears to be double the volume of individual nucleoli of sister karyomeres. As PÄTAU (1936) has pointed out, the fusion of karyomeres probably is to be considered a consequence of the progressive decrease in cross-sectional area of the spindle concurrent

with advancing cleavage and resultant crowding of the vesiculating chromosomes at the poles. By the close of the ninth cleavage karyomery is virtually absent at interphase. In all succeeding cleavages, characterized by small biacuminate spindles, the interkinetic nucleus is a single vesicle.

4. Mitosis of Embryogenesis. At the tenth cleavage of the egg and the succeeding divisions of the embryo the course of mitosis is quite orthodox. The resting nucleus is a single spherical vesicle (fig. 98). Although in the late embryo the resting nucleus generally possesses but one nucleolus, in the young embryo there may be as many as four nucleoli within a single nucleus. In prophase the chromosomes condense about the periphery of the nucleus (fig. 99), and, foreshadowing the appearance of the spindle, the late prophase nucleus is elongated (fig. 100). At metaphase the chunky chromosomes form a regular plate at the equator of a biacuminate spindle (fig. 102). In polar view the metaphase plate is observed to consist of a ring of five chromosomes encircling a central member (fig. 101). In anaphase the chromosomes appear to undergo parallel displacement and there seems to be a pronounced elongation of the interchromosomal spindle (figs. 103, 104). As a rule no equatorial bodies are to be found in any of the mitoses following the ninth cleavage of the egg. As implied by this description of the mitoses of late cleavage and embryonic development, REUTER's (1909b) account of these divisions is in error.

5. Critique of Karyomerokinesis. In evaluating REUTER's (1909b) account of the mitosis of early cleavage in *Pediculopsis*, it must be borne in mind that REUTER employed the best methods of the period in which he worked. Despite his care, fixatives such as Carnoy, Perenyi and Brucker are eminently unsuited to this material. These fluids, however, gave his best preparations. It is a curious and unfortunate coincidence that the egg of *Pediculopsis*, characteristically possessing chromosomal vesicles during its non-dividing stages, should undergo vesiculation of its early cleavage spindles when treated with Carnoy and Perenyi solutions. Thus there was no stage of early cleavage mitosis in which vesiculoid structures were absent in REUTER's slides. As vesicles appeared to be present in all stages of mitosis, REUTER's description of *Karyomerokinesis* seems the simplest interpretation of his slides.

REUTER correctly noted that each chromosome of early cleavage interphase customarily possesses its own nucleus and that the chromosomal vesicle elongates during prophase. However, his metaphase and anaphase karyomeres in reality are rifts in the spindle effected by the distorting action of unsuitable fixatives, hence artifacts. The individual spindles are likewise probably artifacts *(vide ut supra)*. The chromosomoids of REUTER's figures 4 and 5 (fig. 62f) are in reality metaphase chromosomes at a stage prior to their splitting, and figure 7b (fig. 62h) seems to be a polar view of an haploid metaphase plate. REUTER's

conclusions that the chromosomoids are entirely unrelated to the chromosomes made possible and furthered his erroneus estimate of the chromosome garniture of *Pediculopsis* as four. It is of interest to note that REUTER specifically states: „Ich habe in einigen Blastomeren sechs auf diese Weise sehr regelmäßig angeordnete Körper (= chromosomoids, *hence chromosomes*) gesehen ....." The chromosomoid of REUTER's figure 6 (fig. 62g), however, is the *"equatorial body"* of the present paper, while the supposed rows of degenerating ends of spindle fibers of this figure are actually sister chromosomes in early anaphase. The achromatic chromosomes of all of REUTER's stages of *Merokinesis* appear to be stringy coagula of divers nature. In the prophase they may represent understained chromosomes, whereas in the anaphases (REUTER's *metanaphase*) they seem to be warped and misplaced spindle fibers more centrally located in the artifact rifts in the spindle. The chromatic blobs of early telophase (fig. 62i) apparently represent misinterpretations of optical sections of telophase chromosomes. The chromatic granules of the interphase chromosomal vesicles, on the other hand, are nucleoli (fig. 62 j, k). The degeneration of the chromosomoids is a real phenomenon, but the bodies of his figure 12 (fig. 62k) seem to be fixation artifacts. With poor fixation such strongly staining bodies appear on the surface of the spindle.

In conclusion it must be stated that the validity of these interpretations of *Merokinesis* have been checked by personal study of three of REUTER's original slides of *Pediculopsis*, with which he most generously furnished me.

6. The Equatorial Bodies or Chromosomoids. It is evident that the early cleavage divisions of *Pediculopsis graminum* REUT. are rendered unique among known somatic mitoses by the constant elaboration between separating sister chromosomes of a body that strikingly simulates a chromosome. But aside from this startling attribute of cleavage, the mitotic phenomena during early segmentation of the egg of *Pediculopsis* appear to agree in detail with those of *Pediculoides* so admirably presented by PÄTAU (1936)[1]. In comparing the interphase karyomeres of these two mites, however, one further difference may be noted. In *Pediculopsis* there invariably is present a Feulgen-negative nucleolus within each karyomere, whereas it would seem from PÄTAU's account that *Pediculoides* has karyomeres devoid of conspicuous nucleoli. The cleavage mitoses of *Tetranychus* (SCHRADER 1923) conform essentially to those of *Pediculoides* and *Pediculopsis*. Unlike *Pediculopsis*, however,

[1] Through the kindness of Dr. KLAUS PÄTAU I have had the pleasure of examining a number of his beautiful preparations of *Pediculoides*. Among the slides examined were preparations which had been restained with Heidenhain following Feulgen. Unlike *Pediculopsis*, Heidenhain restaining disclosed no suggestion of equatorial bodies in early cleavage anaphases of *Pediculoides*.

the mitosis of *Tetranychus* is not complicated by equatorial bodies[1]. Nevertheless, it would appear that the chromosomal vesicles of *Tetranychus* possess nucleoli as in *Pediculopsis*. The ellipsoidal spindle seems to be the principle distinguishing characteristic of early cleavage mitosis in *Tetranychus*. The mitoses during embryonic development of *Pediculopsis* and *Tetranychus* are much the same, and *Pediculoides* differs from these only in the described absence of a nucleolus during its interphase.

However, in the Lepidoptera (SEILER 1914, 1923; DONCASTER 1922; KAWAGUCHI 1928; and FOGG 1930) and Trichoptera (KLINGSTEDT 1931, 1932) there is an elimination of material in the first maturation division of the egg which *appears* to be strikingly like the elaboration of *Pediculopsis*. BAUER (1932, 1933) has shown that in *Ephestia* the eliminated material is negative to Feulgen; such is probably also the case for the other Lepidoptera and the Trichoptera. Although BĚLAŘ (1928) and GEITLER (1934) believe that during the first maturation division of the egg of the coelenterate *Haliclystis* there is an elimination process similar to that of the Lepidoptera, WEILL, the investigator of this form, described no such phenomenon. Indeed, WEILL (1924) claimed that the bodies at the equator of the spindle were formed as thickenings of the spindle fibers. His poor figures and trifling description allow but slight support to BĚLAŘ's and GEITLER's interpretation, and indeed no more to his own.

v. HAFFNER (1922b) has likewise concluded that the bodies in the equator of the first maturation spindle of *Armillifer* are thickenings of the spindle fibers. In the Porifera, DUBOSCQ and TUZET (1937) have recorded the appearance of siderophil materials between the separating anaphase chromosomes in the first maturation division of the egg of *Grantia* and in both maturation divisions of the egg of *Sycon*. The siderophil bodies they regard as «... épaississements allongés des fibres fusoriales» or «... les fibres interzonales ... qui s'imprégneraient seulement de chromatine». These bodies or thickenings are held to be «chromatine d'élimination» *(Sycon)* or «chromatine de diminution» *(Grantia)*. As DUBOSCQ and TUZET cite no satisfactory evidence that the material is in the form of spindle fiber thickenings, or that it is diminuted or eliminated from the chromosomes, or even that it is chromatin, the cases of *Grantia* and *Sycon*, along with *Haliclystis* and *Armillifer*, remain *incertae sedis*[2]. The peculiar equatorial rings of the generative nuclear division of

[1] It has been my pleasure to ascertain this by study of Professor FRANZ SCHRADER's excellent slides of *Tetranychus*. — [2] The presence of these "mid-bodies" between separating chromosome plates in early anaphase in all four of these cases, however, makes it extremely unlikely that they are "focalization bodies" brought about by advancing division furrows. They may be added to FRY's (1937) class of "atypical mid-bodies", i. e., — mid-bodies or thickenings of the spindle which appear in the absence of focalization. So defined, atypical mid-bodies constitute an aggregate apparently much larger and less rare than FRY has supposed.

the fungus *Basidiobolus lacertae*, described by LOEWENTHAL (1903), may represent elimination materials. But again no conclusions may be reached from the recorded data.

Pediculopsis stands apart from all other cases in that there are at least eleven consecutive division cycles (513 karyokineses) during which equatorial bodies are elaborated. The close correspondance of these eleven division cycles, in every detail but elaboration, with those of *Pediculoides* (PÄTAU 1936), seems to show that only experimental treatment can bring about an elucidation of the nature of the elaboration process in *Pediculopsis*. To ascribe the cause of elaboration to the known differences between these two species is to redescribe these differences in an unwarranted manner. To seek similarities of *Pediculopsis* to the Lepidoptera and Trichoptera to explain the occurrence of equatorial bodies would appear to be productive only of fallacious argumentation. While BAUER (1933) seems to be correct in denying that the elimination of *Ephestia* is correlated with regulation of the nucleo-plasmatic ratio (as SEILER maintains for other moths), he also states that in *Ephestia:* „Es handelt sich bei der Elimination wahrscheinlich nur um eine Abgabe überflüssiger Stoffe, die während der Verkürzung der Tetraden noch (teilweise) in ihnen verblieben sind und nun nachträglich ausgestoßen werden." Although this may be the correct explanation, the problem requires experimental treatment.

VIII. Notes on the Chromosomes of Pediculopsis.

It has been shown above that each vesiculating chromosome of telophase, during the course of formation of an interkinetic vesicle, produces a Feulgen-negative nucleolus (figs. 50, 59). Such a nucleolus is present in each chromosomal vesicle at each stage (excepting late prophases) during which karyomery prevails (figs. 59, 60, 65, 79, 95, etc.). Although vague, but limited, dark-staining patches have been observed in but a few chromosomes of such an early stage of vesiculation as that at which the chromosomes appear to be slender tuber (fig. 78), the formal demonstration of the origination of the nucleolus on the chromosome is to be found in a figure just prior to tubulization. At late anaphase or early telophase (fig. 77, upper), each chromosome may show a darkly staining nucleolus-like body submedially on its length. Such an early telophase figure strongly recalls those of HEITZ (1931) for *Vicia faba*, and would suggest that each of the chromosomes of *Pediculopsis* is divided into two unequal arms by a secondary constriction (HEITZ 1935). However, if such be the case, the secondary constrictions of *Pediculopsis* must be active at a stage as early as the elaborative phase, during which the chromosomoid is formed and but little anaphasic movement has been effected, for the destained chromosomoids corresponding with the

chromosomes of this figures likewise possess single foci resistant to stain extraction (fig. 77, lower).

The inference concerning the achromatic constriction is realized, for the prometaphase chromosome may show two very unequal arms separated by an achromatic constriction (fig. 88). It was shown above that the half-bivalents of diakinesis likewise possess two very unequal arms (figs. 19—21), as well as an achromatic region intercalated between them (fig. 20, lower right). These arms must shorten differentially for the metaphase bivalent and anaphase univalent are symmetrical structures possessing medial achromatic constrictions (see page 64; figs. 42, 44). Such also is the case during early cleavage. Just prior to the formation of the equatorial plate the chromosome possesses two nearly equal arms (fig. 89); at metaphase, during which greatest linear contraction of the chromosome is realized, the demonstrable achromatic constrictions are found to be medially located on the chromosomes (fig. 93). These achromatic constrictions as such are demonstrable only rarely, and have been noted only in Navashin-Feulgen preparations. Restaining of successful preparations with Heidenhain gives figures in which the detail of the achromatic constriction is obliterated. The figures of SCHRADER (1923) for *Tetranychus* suggest that this mite also possesses chromosomes divided into two arms. It is of some interest, therefore, to point out that the interkinetic karyomeres of *Tetranychus* likewise appear to possess nucleoli. Presumably, such nucleolar constrictions are absent from the chromosomes of *Pediculoides*, for, as noted above, the karyomeres apparently do not possess nucleoli (PĂTAU 1936).

In a small number of figures one or more chromosomes of cleavage cells in *Pediculopsis* seem to possess a globular segment or trabant at one extremity (figs. 70, 85, 92). Whereas the majority of the chromosomes of the metaphase figures of one haploid egg appear to possess trabant-like appendices (fig. 94), no indications were noted which pointed to a partic ular differential arm of the chromosomes. Indeed, unlike the secondary constriction with its inevitably attendant interkinetic nucleolus, the trabents of the chromosomes of *Pediculopsis* must be viewed with reserve.

No indication of a kinetochore has been found in any of the chromosomes of *Pediculopsis*. Nor is there any reason for associating the „trabant" described above with such a structure. Presumably the moving force during late anaphase of the first maturation division is directed chiefly at one end of each of the separating univalents, for anaphase figures show that one end of each univalent preceeds to the pole (figs. 35, 39, 40). Although it was not possible to ascertain the configurations of the separating chromosomes during the anaphase of the second maturation division, it is not unthinkable that the *ends* of these chromosomes preceed the mid-regions during poleward movement (see page 70; figs. 46, 47, 53). In the cleavage divisions the elongated chromosomes undergo

parallel displacement (figs. 74—76, 80, 81). PĂTAU (1936) has discussed the movements of the elongated chromosomes of cleavage in *Pediculoides*, and in the absence of new details disclosed by *Pediculopsis* further argumentation seems unwarranted.

In the discussion of maturation prophase (see page 64), it was pointed out that the diakinetic bivalents can hardly be conceived to be physically held together by chiasmata. During diakinesis the two split halves of each bivalent are considerably removed from one another (figs. 19—21), and no exchange of chromatids between the separated partners is evident. A confession of ignorance must be made concerning the nature of origin of these non-chiasmate diakinetic bivalents, but it seems of some interest to indicate here the possible means by which such a condition might arise. Assuming (on the basis of those diplotene figures in which analysis could be made) two chiasmata per bivalent, four possibilities seem outstanding: 1. the chiasmata may be completely terminalized but terminal adhesion of the members fail to occur, 2. the chiasmata may terminalize at the same end of the bivalent and by cancelling each other free the members of the bivalent, 3. the chiasmata may move towards one another (or one move and the other remain stationary) and by cancelling each other as in (2) free the homologs, and 4. the diplotene points of exchange (figs. 14, 15) may not be chiasmata but be nothing more than twists of the homologs about one another.

Now the first possibility seems unlikely for, if such were the case, it would be expected that the diakinetic bivalents would appear as disjoined half-rings. Briefly, each half-bivalent would be expected to be U-shaped and the free ends of each pair of U's would be most approximated. Such figures have not been observed.

The second and third possibilities necessitate constant reciprocal exchange of partners at the two points of crossing-over. Such a condition of invariable, determinate reciprocal exchange in crossing-over is not known in any organism[1]. If such a condition exists in *Pediculopsis*, however, passage of both chiasmata to one and the same end should result (2a) in dissociated bivalents consisting of two straight rods most closely associated at one end while the other ends would be widely divergent. If, albeit (2b), the proximal chiasma moved at such a rate that it invariably caught up with the distal chiasma and cancelled the latter in a subterminal position, figures essentially like those found (fig. 19) would result. The third possibility is also complex, but likewise would result in configurations similar to those actually observed (fig. 19). Movement of chiasmata *away* from the ends of the chromosomes has been suggested by WHITE (1936) to account for pseudolocalization in *Allium* (LEVAN

[1] DARLINGTON (Genetics 19, 1934) has inferred such a condition for the sex chromosomes of *Drosophila*; see also his remarks on *Fritillaria* and *Aggregata*.

1933, 1935) and *Anemone* (MOFFETT 1932). But there seems no good reason for preferring alternative (3) to (2b).

The fourth possibility — namely, that there are no chiasmata in the meiotic prophase of the egg of *Pediculopsis* — seems unlikely for it precludes the possibility of crossing-over in this organism as the males are haploid (COOPER 1937). Final answer concerning this fourth possibility lies in breeding experiments. It will be observed that hypotheses based upon one or three (or more) chiasmata require conditions that have not been found in the cytologic preparations, or assumptions for which there exists no evidence.

Insofar as may be judged, the condensed bivalents of the egg of *Tetranychus* (*see* SCHRADER 1923, fig. 1) may be devoid of chiasmata and laterally associated like those of *Pediculopsis*. The bivalents of *Pediculoides*, on the other hand, presumably result from complete terminalization of chiasmata (*see* PÄTAU 1936, figs. 8h, i; 9a, b, c, e), and take the form of rods or rings depending upon the number of chiasmata involved. While such an interpretation of the bivalents of *Pediculoides* appears satisfactory, it is by no means adequately supported by detailed data.

Whereas there are indications of an unusual structure of the bivalents in the spermatocytes of *Argas*, OPPERMANN's (1935) figures and description seem too inadequate for interpretation of the organization of the tetrads. Furthermore, the compound of X and Y chromosomes in the male of *Argas* must be viewed with reserve. Only more convincing and later stages of the anaphase of the second maturation division than that of OPPERMANN's figure 14 can show that the appendage to the X is really disjoined and segregated as the Y-chromosome.

In the Parasitids investigated by SOKOLOFF (1934), however, there appears to be a marked tendency for bivalents to be dissociated in the oöcyte-like phase of „großen Wachstums[1]" of the spermatocytes following diakinesis. SOKOLOFF claims to have observed chiasmata in diplotene and diakinetic chromosomes of Parasitid spermatocytes, but holds that the chiasmata are completely terminalized by the onset of the second, exaggerated growth stage of the spermatocyte. In this latter phase not only do homologous half-bivalents appear to be separated from each other, but their component chromatids likewise seem to be disjoined. While SOKOLOFF has not obtained a close series of stages from late diakinesis through anaphase of the first maturation division of these mites, he states „... daß auch im Verlauf der ganzen 1. Reifungsteilung der Zusammenhang der Chromatiden fast immer mehr oder weniger stark gelockert bleibt". It is quite possible that the separated half-bivalents of these spermatocytes of „großem Wachstum" in *Gamasus* express in exaggerated degree the peculiar condition here described in *Pediculopsis*. SOKOLOFF, however, has attributed balances of forces of repulsion and affinity to

[1] OPPERMANN (1935) likewise records a second growth period, recalling oögenetic processes, in the developing spermatocytes of *Argas*.

account for the symmetrical figures of dissociated bivalents that he has observed. In actuality this currently popular mode of explanation is hardly more than an unjustifiable mechanical redescription of the observed cytologic figures. It must be kept ever in mind that the data emergent from investigations such as those of SOKOLOFF and the present writer can only concern the morphologic and static properties of the chromosomes. For this reason the thread-like structures running between the apices of the half-bivalents of *Pediculopsis* at diakinesis (fig. 20) awaken some interest. These threads, however, are observed only between the halves of two of the bivalents of this figure and were not observed in any other preparations. Their unique occurrence and the fact that they appear similar to the non-staining coagulum of the nucleus of this figure suggest that they are artifacts. The tenuity and staining properties of the threads likewise point to the conclusion that these threads are not segments of the chromatids of the bivalents. Lastly, these fibers do not appear to be related to the thread-like structures present between half-bivalents during early elaboration (fig. 31). They are temporally separated from the latter by a considerable period, all observed bivalents of which appear to be devoid of such structures. If, for the sake of argument, it be granted that such threads *do* hold the apices of the half-bivalents in approximation, it can hardly be conceived that they are responsible for the symmetrical disposition of homologous half-bivalents in a plane.

A non-staining halo may be perceived to surround each bivalent during diakinesis (figs. 21, 22) and metaphase (figs. 28—32). Similar sharply delimited halos may be observed to enclose each univalent of the second maturation division during the elaborative phase (fig. 51), as well as enclosing sister chromosomes during cleavage metaphase (figs. 71, 72). It may be that the nimbous areas about the chromosomes of these stages are sheaths in the sense of METZ (1934), and are responsible for maintaining the accurate alignment of sister half-bivalents and the parallel disposition of sister chromosomes which have undergone but modest displacement during the elaborative phase of early cleavage. Pending demonstration that these halos or "sheaths" are something more than pure artifacts — for they also appear about the equatorial bodies of the first maturation division during early anaphase (figs. 37, 43) — the problem of the collocating mechanism must remain an open one.

Summary.

1. Examination of three of REUTER's original slides demonstrated that the material upon which this investigation is based is identical with REUTER's species. *Pediculopsis graminum* (REUT.) has a diploid number of six, not four as REUTER claimed.

2. Whereas early diplotene bivalents of the oöcyte appear to possess chiasmata, the bivalents at diakinesis and metaphase are not physically held together by chiasmata nor any other visible structure.

3. Feulgen-negative bodies are elaborated between separating chromosomes in both maturation divisions of the egg and correspond to the chromosomes in both number and position. These bodies degenerate at the equatorial plate.

4. Entry of the spermatozoön occurs during the first maturation metaphase of the egg. The male pronucleus has the form of a single, trilobed vesicle, and does not undergo fusion with the three karyomeres of the female pronucleus.

5. Each chromosome possesses an individual nucleus or karyomere during early cleavage of the egg. In prophase the karyomere, within which the chromosome condenses, elongates and ultimately disappears.

6. During cleavage the chromosomes first show evidences of a longitudinal split at late metaphase. Between the separating chromosomes a Feulgennegative equatorial body is formed; the latter degenerates in telophase.

7. During cleavage the chromosomes undergo parallel displacement in anaphase; at telophase each chromosome forms an elongate vesicle which becomes a spherical karyomere at interkinesis.

8. Karyomery and formation of equatorial bodies cease by the tenth cleavage division, and mitoses of early and late embryos and of oögonia are normal.

9. It is shown that REUTER's description of a *Karyomerokinesis* during cleavage divisions of *Pediculopsis graminum* (REUT.) is based upon misinterpretation of the mitotic figures. The validity of the reinterpretation of REUTER's findings presented here has been checked by personal study of three of REUTER's original slides.

10. Chromosomes length appears to be limited and controlled by cross-sectional area of the metaphase spindle during cleavage.

11. Each chromosome of *Pediculopsis* possesses a secondary constriction concerned with nucleolus formation. The two arms of each chromosome appear to shorten differentially during meiotic and cleavage prophases.

12. A trabant may terminate each chromosome of *Pediculopsis*, but there is no reason to associate the functions of a kinetochore with this structure.

Zusammenfassung.

1. Die Untersuchung von drei Präparaten REUTERS zeigt, daß das Material, an welchem die jetzige Untersuchung vorgenommen wurde, mit REUTERS Art identisch ist. *Pediculopsis graminum* (REUT.) hat die diploide Zahl 6, und nicht 4, wie REUTER angab.

2. Während Bivalente im frühen Diplotän der Oöcyte Chiasmata zu haben scheinen, werden die Bivalenten der Diakinese und Metaphase

weder durch Chiasmata noch irgendeine andere sichtbare Struktur zusammengehalten.

3. Feulgen-negative Körper werden im Ei in beiden Reifungsteilungen zwischen den auseinanderrückenden Chromosomen gebildet und entsprechen in Anzahl und Stellung den Chromosomen. Diese Körper verbleiben im Äquator und degenerieren.

4. Das Spermatozoon dringt während der Metaphase der ersten Reifungsteilung in das Ei ein. Der männliche Vorkern hat die Gestalt eines Bläschens mit drei Ausbuchtungen; er verschmilzt nicht mit den drei Karyomeren des weiblichen Vorkerns.

5. In den frühen Furchungsteilungen bildet jedes Chromosom ein Karyomer, das sich während der prophasischen Chromosomenbildung verlängert und schließlich verschwindet.

6. Während der Furchung wird der Längsspalt der Chromosomen in der späten Metaphase bemerkbar. Zwischen den auseinanderrückenden Chromosomen wird ein Feulgen-negativer Körper wie in den Reifungsteilungen gebildet; dieser degeneriert in der Telophase.

7. In den Furchungsanaphasen behaupten die Chromosomen eine senkrechte Stellung zur Spindelachse; in der Telophase bildet jedes Chromosom ein längliches Bläschen, welches in der Interkinese zum runden Karyomer wird.

8. Die Bildung von Karyomeren sowie der äquatorialen Körper hört in der zehnten Furchungsteilung auf, und die Teilungen im Embryo und in den Oögonien sind normal.

9. Es wird gezeigt, daß Reuters Beschreibung der *Karyomerokinesis* während der Furchungsteilungen auf einer Fehldeutung der Teilungsfiguren beruht. Die Gültigkeit der bisher gegebenen Interpretation ist durch das Studium von Reuters eigenen Präparaten bestätigt worden.

10. Die Chromosomenlänge während der Furchung scheint durch den Spindelquerschnitt begrenzt und bedingt zu sein.

11. Jedes Chromosom besitzt eine sekundäre Einschnürung, die mit der Nukleolusbildung zu tun hat. Die hierdurch gebildeten beiden Abschnitte jedes Chromosoms scheinen sich während der mitotischen und Furchungsprophase verschieden weitgehend zu verkürzen.

12. Es ist möglich, daß jedes Chromosom in einem Trabanten endigt, doch besteht kein Grund, diesem die Tätigkeit eines Kinetochors zuzuschreiben.

Literature cited.

Amerling, C.: Die Milbenkrankheit unserer Getreidearten. Lotus **11** (1861). — **Bauer, H.:** Die Feulgensche Nuklealfärbung in ihrer Anwendung auf cytologische Untersuchungen. Z. Zellforsch. **15** (1932). — Die wachsenden Oocytenkerne einiger Insekten in ihrem Verhalten zur Nuklealfärbung. Z. Zellforsch. **18** (1933). — **Bělař, K.:** Die cytologischen Grundlagen der Vererbung. Handbuch der Vererbungslehre, Bd. 1. 1928. — **Bonnet, A.:** Sur les organes génitaux mâles et la spermatogenèse

chez les Ixodes. C. r. Assoc. France avanc. Sci. Lyon **35** (1906). — Recherches sur l'anatomie comparée et le développement des Ixodidés. Ann. Univ. Lyon, N. s. 1, Sci. **20** (1907). — **Bradbury, O. C.:** Some phases of the embryology of the carnation mite. J. Elisha Mitchell Sci. Soc. **42** (1926). — **Casteel, D. B.:** Cytoplasmic inclusions in male germ cells of the fowl tick, *Argas miniatus*, and histogenesis of the spermatozoon. J. Morph. a. Physiol. **28** (1917). — **Claparède, E.:** Studien an Acariden. Z. Zool. **18** (1868). — **Christophers, S. R.:** The anatomy and histology of ticks. Sci. Mem. Off. Med. Sanit. Dept. Gov. India, N. s. **1906**, Nr 23. — **Cooper, K. W.:** Reproductive behavior and haploid parthenogenesis in the grass mite, *Pediculopsis graminum* (Reut.) (Acarina, Tarsonemidae). Proc. nat. Acad. Sci. U.S.A. **23** (1937). — **Darlington, C. D.:** The internal mechanics of the chromosomes. I. The nuclear cycle in *Fritillaria*. Proc. roy. Soc. Lond. B **118** (1935). — Recent advances in cytology, 2nd Ed. Philadelphia 1937. — **Doncaster, L.:** Further observations on chromosomes and sex-determination in *Abraxas grossulariata*. Quart. J. microsc. Sci. **66** (1922). — An introduction to the study of cytology. Cambridge 1924. — **Duboscq, O.** et **O. Tuzet:** L'ovogenèse, la fécondation et les premiers stades du développement des éponges calcaires. Archives de Zool. **79** (1937). — **Ewing, H. E.:** The suborders and superfamilies of Acarina. Proc. helminth. Soc. Wash. **1** (1934). — **Fogg, L. C.:** A study of chromatin diminution in *Ascaris* and *Ephestia*. J. Morph. a. Physiol. **50** (1930). — **Fry H. J.:** Studies of the mitotic figure. VI. Mid-bodies and their significance for the central body problem. Biol. Bull. Mar. biol. Labor. Wood's Hole **73** (1937). — **Geitler, L.:** Grundriß der Cytologie. Berlin 1934. — **Gilson, G.:** Étude comparée de la spermatogénèse chez les arthropodes. 3 P. Acariens. Cellule **4** (1888). — **Haffner, K. v.:** Beiträge zur Kenntnis der Linguatuliden. I. Ovarium und Eibildung von *Porocephalus armillatus* (Wyman). Zool. Anz. (1922a). — II. Zur Eireifung von *Porocephalus armillatus* (Wyman). Zool. Anz. (1922b). — **Heitz, E.:** Nukleolen und Chromosomen in der Gattung *Vicia*. Planta (Berl.) **15** (1931). — Die Herkunft der Chromocentren. Planta (Berl.) **18** (1933). — Chromosomenstruktur und Gene. Z. Abstammgslehre **70** (1935). — **Heller, C.:** Zur Anatomie von *Argas persicus*. Sitzgsber. Akad. Wiss. Wien **30** (1858). — **Henking, H.:** Beiträge zur Anatomie, Entwicklungsgeschichte und Biologie von *Trombidium fuliginosum* Herm. Z. Zool. **37** (1882). — **Heymons, R.:** *Pentastomida*. Kükenthals Handbuch der Zoologie, Bd. 3 (1926). — **Hughes-Schrader, S.:** A study of the chromosome cycle and the meiotic division-figure in *Llaveia bouvari* — a primitive coccid. Z. Zellforsch. **13** (1931). — **Kawaguchi, E.:** Zytologische Untersuchungen am Seidenspinner und seinen Verwandten. Z. Zellforsch. **7** (1928). — **Klingstedt, H.:** Digametie beim Weibchen der Trichoptere *Limnophilus decipiens* Kol. Acta zool. fenn. **10** (1931). — Släktskapen Trichoptera-Lepidoptera i cytologius ljus. Notulae Ent. **11** (1932). — **Knaben, N.:** Oogenese bei *Tischeria angusticolella* Dup. Z. Zellforsch. **21** (1934). — **Koch, A.:** Morphologie des Eiwachstums der Chilopoden. Z. Zellforsch. **2** (1925). — Studien über den Dotterkern der Spinnen. Z. Zellforsch. 8 (1929). — **Kraczkiewicz, Z.:** Contribution à l'étude du «noyau vitellin» chez *Cylindroiulus londinensis* (Diplopoda). C. r. Assoc. Anat. Varsovie **1931**. — **Levan, A.:** Cytological studies in *Allium*. IV. *Allium fistulosum*. Sv. bot. Tidskr. **27** (1933). — VI. The chromosome morphology of some diploid species of *Allium*. Hereditas (Lund) **20** (1935). — **Leydig, F.:** Zum feineren Bau der Arthropoden. Arch. Anat., Physiol. u. wiss. Med. **1855**. — **Loewenthal, W.:** Beiträge zur Kenntnis des *Basidiobolus lacertae* Eidam. Arch. Protistenkde **2** (1903). — **Metz, C. W.:** The rôle of the "chromosome sheath" in mitosis, and its possible relation to phenomena of mutation. Proc. nat. Acad. Sci. U.S.A. **20** (1934). — **Michael, A. D.:** British Oribatidae, Vol. 1. Ray Soc. Lond. **1884**. — On the variations in the internal anatomy of the Gamasinae, especially in that of the genital organs, and on their mode of coition. Trans. Linn. Soc. Lond., II. s. **5** (1892). — The internal anatomy of *Bdella*. Trans. Linn. Soc. Lond., II. s. **6** (1896). — **Moffett, A. A.:** Studies in *Anemone*. I. A new type of chiasma behavior.

Cytologia 4 (1932). — **Molz, E. u. O. Morgenthaler:** Die Sporotrichium-Knospenfäule, eine für Deutschland neue Nelkenkrankheit. (Zugleich ein Fall von Symbiose.) Ber. dtsch. bot. Ges. **30** (1912). — **Nordenskiöld, E.:** Zur Spermatogenese von *Ixodes reduvius*. Zool. Anz. **34** (1909a). — Zur Ovogenese und Entwicklungsgeschichte von *Ixodes reduvius*. Zool. Anz. **35** (1909b). — Spermatogenesis in *Ixodes ricini* Linn. Parasitology **12** (1920). — **Oppermann, E.:** Die Entstehung der Riesenspermien von *Argas columbarum* (Shaw) (*reflexus* F.). Z. mikrosk.-anat. Forsch. **37** (1935). — **Pagenstecher, H. A.:** Beiträge zur Anatomie der Milben. H. 1. Leipzig 1860. — Beiträge zur Anatomie der Milben, H. 2. Leipzig 1861. — **Pätau, K.:** Cytologische Untersuchungen an der haploid-parthenogenetischen Milbe *Pediculoides ventricosus*, Newp. Berl. zool. Jb. **56** (1936). — **Reuter, E.:** Über die Weißährigkeit der Wiesengräser in Finnland. Acta Soc. proc. Fauna et Flora fenn. **19** (1900). — Über die Eibildung bei der Milbe *Pediculopsis graminum* (E. Reut.). Festschr. f. Palmén, Nr 7 1907. — Zur Morphologie und Ontogenie der Acariden. Acta Soc. Sci. fenn. **36** (1909a). Merokinesis, ein neuer Kernteilungsmodus. Acta Soc. Sci. fenn. **37** (1909b). — Några ord om hvitaxacariden *Pediculopsis (Pediculoides) graminum* E. Reut., dess geografiska utbredning och dess förekomst tillsammans med en *Sporotrichium*-Art. Helsingfors Medd. Soc. Fauna et Flora fenn. **35** (1909c). — Beiträge zu einer einheitlichen Auffassung gewisser Chromosomenfragen. Acta zool. fenn. **9** (1930). — **Sambon, L. W.:** A synopsis of the family *Linguatulidae*. J. trop. Med. **25** (1922). — **Samson, K.:** Zur Spermiohistiogenese der Zecken. Sitzgsber. Ges. naturforsch. Freunde Berl. 8 (1909). — **Schmidt, U.:** Beiträge zur Anatomie und Histologie der Hydracarien, besonders von *Diplodontus despeciens* O. F. Muller. Z. Morph. u. Ökol. Tiere. **30** (1935). — **Schrader, F.:** Haploidie bei einer Spinnmilbe. Arch. mikrosk. Anat. **97** (1923). — **Seiler, J.:** Das Verhalten der Geschlechtschromosomen bei Lepidopteren. Arch. Zellforsch. **13** (1914). — Geschlechtschromosomen-Untersuchungen an Psychiden. IV. Z. Abstammgslehre **31** (1923). — **Sokoloff, L.:** Untersuchungen über die Spermatogenese bei den Arachniden. V. Über die Spermatogenese der Parasitidae (= Gamasidae, Acari). Z. Zellforsch. **21** (1934). — **Steding, E.:** Zur Anatomie und Histologie von *Halarachne Otariae* n. sp. Z. Zool. **121** (1924). — **Stewart, F. C. and H. E. Hodgkiss:** The *Sporotrichium* bud-rot of carnations and the silver top of June grass. N. Y. agricult. exper. Stat. Techn. Bull. **7** (1908). — **Thor, S.:** Récherches sur l'anatomie comparée des Acariens prostigmatiques. Ann. des Sci. natur., VIII. s. **19** (1904). — **Tuzet, O. and J. Millot:** Récherches sur la spermiogenèse des *Ixodes*. Bull. Biol. de France et Belgique **71** (1937). — **Wagner, J.:** Die Embryonalentwicklung von *Ixodes calcaratus* Bir. (Russian, with German summary.) Trav. Soc. Nat. St. Petersbourg **24** (1894). — **Warren, E.:** On atypical modes of sperm development in certain Arachnids. Ann. Natal Mus. P'm'burg **7** (1933). — **Weill, R.:** La maturation de l'ovule d'*Haliclystis octoradiatus* J. Clark. C. r. Soc. Biol. Paris **90** (1924). — **White, M. J. D.:** Chiasma-localization in *Mecostethus grossus* L. and *Metrioptera brachyptera* L. (Orthoptera). Z. Zellforsch. **24** (1936). — **Winkler, W.:** Anatomie der Gamasiden. Arb. zool. Inst. Wien. **7** (1888). — **Wolcott, R. H.:** A mite accompanying the bud-rot of carnations. Bull. agricult. exper. Stat. Nebr. **20** (1908).

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STRUCTURAL HYBRIDITY IN CIMEX L.

By

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With 37 figures in the text.

(Eingegangen am 29. Dezember 1938).

Introduction.

The diversity of sex chromosome types to be found in the *Hemiptera-Heteroptera* (HARVEY 1916) conveys the impression that the sex mechanism has been subject to extensive changes in the course of the evolution of the present species.

The sub-order embraces species having from one to five X chromosomes with or without a Y chromosome in the heterozygous (male) sex, but in any particular species, the number of its sex chromosomes does not vary.

Only one exception to these completely stabilised sex mechanisms appears to have been reported. In males of three species of *Metapodius*, E. B. WILSON (1909) found that in addition to the X and Y, supernumerary chromosomes may be present. From one to seven of these appeared in different specimens. The specimens on which he worked were obtained from widely separated localities in North America but they were not found to form geographical races with specific numbers of supernumerary chromosomes. In each individual the number of supernumeraries was constant and their behaviour resembled that of the sex chromosomes to which they appeared to be allied.

The present paper on two species of *Cimex* describes a second case in which supernumerary chromosomes occur. In this case their number varies not only in different individual insects but also in different germ cells of the same insect. WILSON found variation in number in all three species of *Metapodius*. In *Cimex* the variation occurs in one of the species, *C. lectularius* L. The second species described, *C. rotundatus* SIGN. shows no variation but forms a useful basis for comparison.

Material and methods.

The material comprises one stock of *Cimex rotundatus* from Liverpool and three stocks of *C. lectularius*, one from laboratory white rats in Edinburgh and two from human sources, one from Glasgow and one from South London. The stocks were kept at room temperature and fed weekly on rabbit's blood. Pair matings were made and offspring reared in an incubator at 32° C. and 90% relative humidity. At this

temperature the life cycle was reduced from the normal length of about 56 days (GUNN 1933) to 36—42 days (PATTON 1931). These bugs were kept in pill-boxes having a hole cut in the bottom covered with bolting silk and were fed every three days on human blood.

The gonads were dissected in 0,6% saline and in CARNOYS fluid, were fixed for 8 hours in LANGLET's Navashin mixture or in ALLEN's Picroformol A3, with prevous treatment in some cases for ten minutes in Carnoy, and embedded by the methyl benzoate-celloidin method (PÉTERFI 1924). Sections were cut 15—20 micra in thickness and stained in NEWTON's Gentian Violet. Camera lucida drawings were made with a $^1/_{16}$" objective and $\times 15$ ocular at table level. Drawings and photomicrographs are reproduced at a magnification of 4400 diameters.

Description.

Observations on the stocks. Because it was thought that the Edinburgh stock (E) of *C. lectularius* differed phenotypically from the Glasgow stock (G) in the ratio of the lengths of the 3rd to the 4th antennal joints the two stocks were originally treated as different races (SLACK 1938b). The examination of a greater number of specimens has shown that, while the mean antennal ratio for stock E is 0.77 and for stock G is 0.70, the maximum and minimum ratios in the two stocks overlap and there appears to be no clear discontinuity between the populations. This is also true of other measurements, such as the mean distance between the outer faces of the eyes and the width of the thorax. On the average these ratios are slightly less in stock G, but the maxima and minima overlap in the same way.

The sex ratio based on a count of 218 individuals in stock E is 1. 24. In pair matings, eight F_1 broods gave the following counts (see table). This series, taken in connection with the ratio in the wild population, shows little evidence of a tendency towards the production of broods of one sex.

	♂♂	♀♀
Brood (1) . . .	8	4
„ (2) . . .	2	2
„ (3) . . .	8	7
„ (4) . . .	1	4
„ (5) . . .	15	2
„ (6) . . .	3	0
„ (7) . . .	0	1
„ (8) . . .	5	3
Total	52	23

Spermatogenesis.

The small size of the nuclei and chromosomes in *Cimex* sp. does not allow a detailed analysis to be made of the early prophase stages of meiosis. Pachytene and early diplotene stages appear to show a similarity to the same stages in *Corixa* (SLACK 1938a) in that the ends of the bivalent threads are fused in heterochromatic masses (Figs. 1, 2). The nature of these masses can only be inferred from the behaviour of the chromosomes at later phases of division.

Nuclei at late diplotene and diakinesis show both bivalents and univalents, the former of a peculiar type (Fig. 3—7). In many bivalents the homologues lie parallel either throughout their length or with the ends diverging, one pair of divergent limbs usually being longer than

the other. In most cases one bivalent forms a cross in which the arms are in the same plane but not at right angles. They often bear small terminal knobs which in some nuclei link one bivalent to another by

Fig. 1. *C. lectularius* ♂ (15)[1] Early Prophase.

Fig. 2. *C. lectularius* ♂ (7). Early Prophase. *c* heteropycnotic body.

fine chromatic threads. With strongly differentiated Gentian Violet staining it is possible to see the split separating the component chromatids.

At first metaphase the bivalent autosomes have contracted to quadripartite structures each part being of an ovoid shape (Fig. 8). They

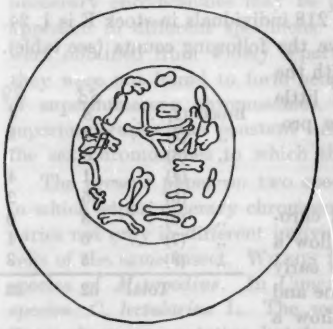

Fig. 3. *C. lectularius* ♂ (12). Late diplotene.

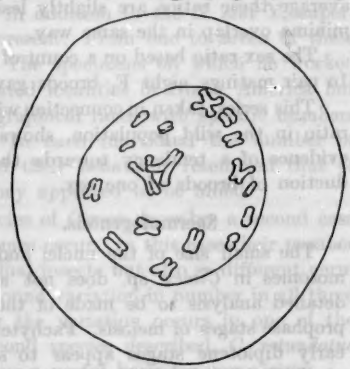

Fig. 4. *C. lectularius* ♂ (5). Late diplotene.

appear to assume the form of U-shaped elements, one arm of the U being shorter than the other in some of the larger bivalents and each arm representing one part of the quadripartite. The ends of the arms are directed towards the poles with the bases of the Us closely apposed

[1] Numbers given in brackets after *C. lectularius* ♂ represent the modal number of univalents found in each specimen.

or in actual contact. Univalents at this stage usually take the form of a pair of sub-spherical or spherical bodies in close apposition and orientated end to end in the spindle axis. The sex chromosome considered

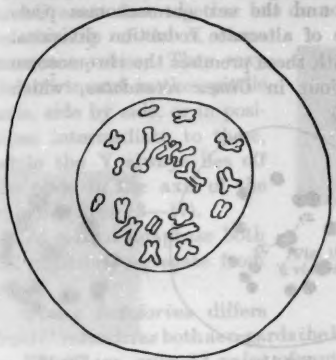

Fig. 5. *C. lectularius* ♂ (5). Late diplotene.

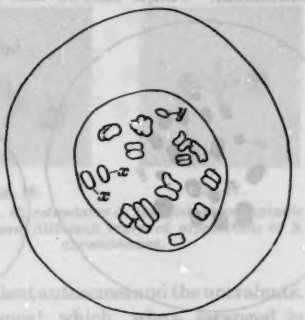

Fig. 6. *C. rotundatus* ♂. Diakinesis.

to be the Y is a univalent exceptional in having a lower degree of contraction and differential staining. It is oblong in form and stains less deeply than any other member of the complement.

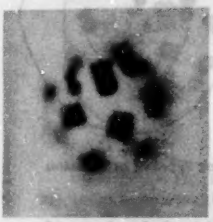

Fig. 7. *C. lectularius* ♂. Diakinesis.

Fig. 8. *C. lectularius* ♂ (9). 1st Metaphase in Spindle view. *a. b.* asymetrical bivalent. *u* univalent.

Fig. 9. *C. lectularius* ♂. 1st Anaphase showing equational division of the Y chromosome.

In order to appreciate chromosome behaviour in *Cimex* it is necessary to bear in mind the sequence of events of general occurrence in the Hemiptera-Heteroptera. The autosomes are paired at metaphase in the primary spermatocyte and their division is reductional, whereas the sex chromosomes are un-paired at this stage and their division is equational (Fig. 9). At second meiotic metaphase the sex chromosomes,

now represented by single chromatids, come close together before separating so that X and Y elements pass to opposite poles at second anaphase, while the autosomes divide equationally. The autosomes are therefore pre-reductional and the sex chromosomes post-reductional. *Cimex* follows this rule of alternate reduction divisions.

With these premises the chromosome behaviour in *Cimex rotundatus*, which

Fig. 10.

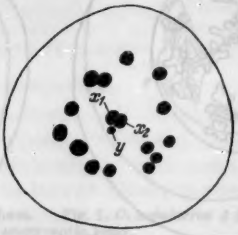

Fig. 11.

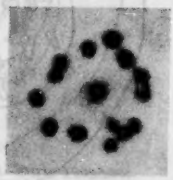

Fig. 12.

Fig. 10. *C. rotundatus* ♂. 1st Metaphase, polar view.
Fig. 11. *C. rotundatus* ♂. 2nd Metaphase, polar view.
Fig. 12. *C. rotundatus* ♂. 2nd Metaphase, polar view.

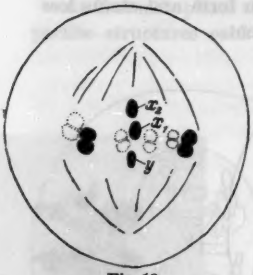

Fig. 13.

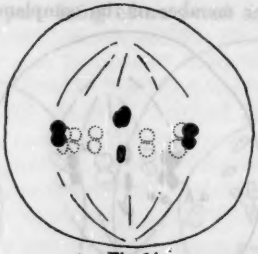

Fig. 14.

Fig. 15.

Fig. 13, 14, 15. *C. rotundatus* Spindle views of 2nd metaphase showing types of orientation of X chromosomes.

appears to be constant, and the remarkably variable behaviour of *C. lectularius* may be described.

In *C. rotundatus* there are 14 pairs of autosomes and three univalent sex chromosomes distributed at random on the 1st metaphase plate (Slack 1938 c) (Fig. 10). While not differing markedly in size one from the other, three groups of autosomes may be distinguished consisting of five large, seven intermediate and two small chromosomes. The sex chromosomes comprise two X elements of similar size to the intermediate autosomes, and one Y chromosome having the characters described and which is smaller than either X.

The second metaphase figure (Figs. 11, 12) shows all autosomes in a ring at the periphery of the plate with the sex chromosomes in a central position, a type of orientation frequent in Heteroptera. X_1, X_2 and Y come close together but do not seem to make contact. Positional relationship of the X chromosomes varies. They may lie end to end in the spindle axis, side by side, or in positions intermediate to these, while the Y always lies off the plate in the axis of the spindle (Figs. 13—17).

Fig. 16.

Fig. 17.

Fig. 16, 17. *C. rotundatus* ♂. 2nd Metaphase, spindle view to show different types of orientation of X chromosomes.

At second anaphase both Xs regularly segregate from the Y.

Cimex lectularius differs from *C. rotundatus* both as regards the bivalent autosomes and the univalents.

There are only 13 pairs of autosomes[1] which, when arranged in order of size, are seen to lack one pair corresponding to one of the large group of *C. rotundatus*.

More important are the differences in the behaviour of the univalents.

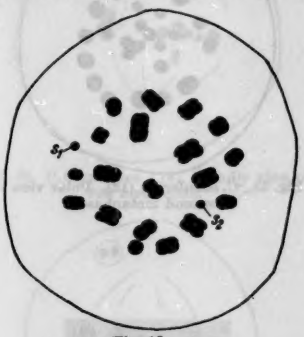

Fig. 18.

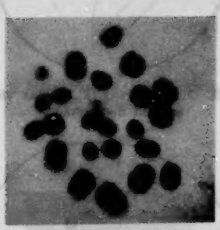

Fig. 19.

Fig. 18. *C. lectularius* (6). 1st Metaphase in polar view showing precocious division of a small univalent S_1 S_2.

Fig. 19. *C. lectularius* ♂. 1st Metaphase.

Instead of the invariable two X chromosomes found in *C. rotundatus* the number of univalent elements varies from 4 to 16. These are recognisable at late diplotene or diakinesis by their structure and at first metaphase are distributed at random on the plate together with the autosomes (Fig. 18, 19). At second metaphase a central group of elements lies

[1] The restriction of the term autosome to these 13 pairs of homologues which form a ring at 2nd metaphase does not imply that all univalents must necessarily be sex chromosomes.

within the ring formed by all the autosomes (Figs. 20—25). In spindle view they are seen to be single spheroidal bodies in contrast to the

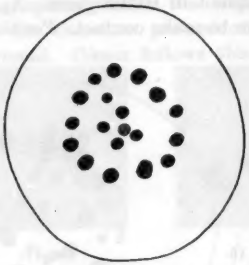

Fig. 20. *C. lectularius* (7). Polar view of second metaphase.

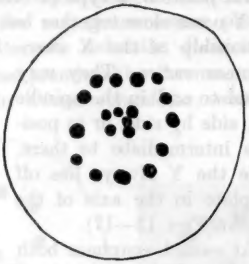

Fig. 21. *C. lectularius* (6). Polar view of second metaphase.

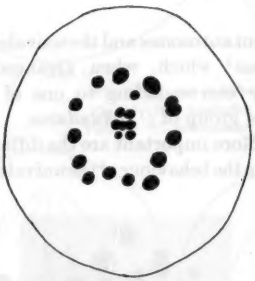

Fig. 22. *C. lectularius* (8). Polar view of second metaphase.

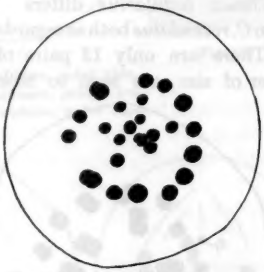

Fig. 23. *C. lectularius* (12). Polar view of second metaphase.

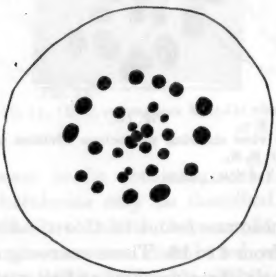

Fig. 24. *C. lectularius* (16). Polar view of second metaphase.

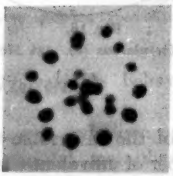

Fig. 25. *C. lectularius* ♂. 2nd Metaphase, polar view.

double structure of the chromosomes in the ring (Figs. 26—31). They represent single chromatids of the univalents seen at the first division. The Y, also a single element resembling that of *C. rotundatus* is

present in every nucleus and may therefore be dis-regarded while considering the variation in number of the central group.

Fig. 26. *C. lectularius* ♂ (5). Spindle view of 2nd metaphase.

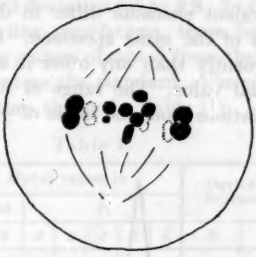

Fig. 27. *C. lectularius* ♂ (7). Spindle view of 2nd metaphase.

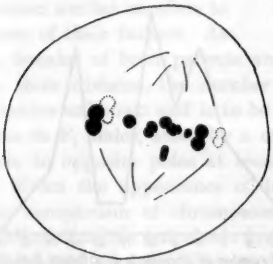

Fig. 28. *C. lectularius* ♂ (8). Spindle view of 2nd metaphase.

Fig. 29. *C. lectularius* ♂ (12). Spindle view of 2nd metaphase.

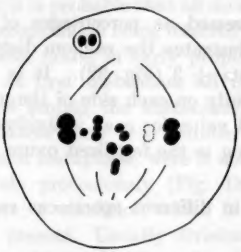

Fig. 30.

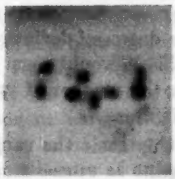

Fig. 31.

Fig 30. *C. lectularius* ♂ (11). Spindle view of 2nd metaphase showing 2 univalents lost from the nucleus at 1st anaphase.

Fig. 31. *C. lectularius* ♂. 2nd Metaphase, spindle view.

This variation in the number of the elements of the central group is the most outstanding feature of meiosis in *C. lectularius*. As both number and form of these chromosomes show the same characters in

the three stocks examined, the stocks may be treated as one population. Ten nuclei in each of 35 individuals have been analysed.

Chromosome counts show that not only do the numbers of central univalent elements differ in different specimens but also in different cells of the same specimen. In most cases one number occurs more frequently than any other in any one individual, giving a well defined modal value. The range of numbers in each individual is limited to deviations from the mode of ± 3, in fact in the great majority of cases to ± 2. Variation within the individual therefore rarely exceeds one third and never more than one half of the total range of variation in the whole population. The frequency of the modal values and

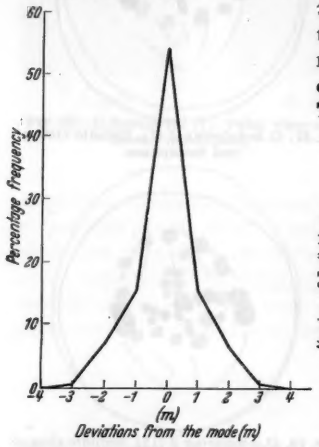

Fig. 32.

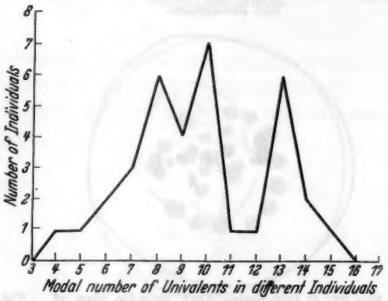

Fig. 33.

deviations from the modal values, expressed as percentages of the total number (364) of nuclei counted, illustrates the random distribution of these deviations within the limits ± 3 (Fig. 32). It is seen that the degree of deviation decreases rapidly on each side of the mode. From this it would seem that the modal value for each individual represents the number of univalents occuring in the fertilized ovum from which it arose.

On this basis the range of numbers in different specimens can be described.

Fig. 33 shows the frequency of modal values (i. e. actual numbers possessed by the individuals) in the population of 35 specimens. The numbers 8—10 and 13 appear to be the most numerous. This however may be due to the relatively low number of specimens examined and it is more likely that there is random distribution within the limits of 4 to 15 in the wild population, since the frequency of numbers of the central group univalents in all the nuclei examined of the whole population

shows random distribution with the mode corresponding to the mean (10) of the range of variation. Taking the modal value 10 as the standard for number of elements in the wild population the mean deviation ($\bar{x}$) from this amounts to 3.5 and the standard deviation (σ) to 3.9. In order to see if the same holds true for pure line populations, these figures may be compared with the corresponding values obtained from their F_1 counts using the mode of the male parent as the standard. Three pair matings (a), (b) and (c) gave modal values shown in Table (1).

In all three pure line stocks the mean and standard deviations are lower than those of the wild population. From this it may be inferred that there is a tendency for F_1 males to possess similar numbers to those of their fathers. As in females of both parents and offspring sections of the ovaries failed to show division, the number of elements contributed by the mothers remains unknown and is to be inferred only from the sequence of numbers in F_1 males, aided by a study of their behaviour when they segregate to opposite poles at second anaphase.

Table 1.

Pair	Modal values in							Deviation fr. parent.	
	Parent		F_1						
	♂	♀	♂	♂	♂	♂	♂	$\bar{x}$	σ
(a)	6	?	10	6	8	7	8	2.50	2.41
(b)	9	?	10	12	9	7	—	1.50	1.87
(c)	8	?	6	7	5	7	—	1.75	1.93

From the appearance of late diplotene and diakinesis figures and the comparison of chromosome counts with those at metaphase it is evident that the univalents are present as such and if all are at any time paired this must be during early prophase when it has not been possible to recognise them. Some of them do appear to be paired at late diplotene and it is probable that all do so at an earlier stage and are heteropycnotic, heteropycnosis being inferred from the presence of the irregular chromatin masses noted in early prophase nuclei.

At first metaphase all univalents appear as separate entities, scattered among the autosomes. This would be the best stage at which to assess their true number, free from the variation which occurs at second metaphase, were it not for the fact that one or more of them may divide precociously (Fig. 18). Their component chromatids then lie apart: a condition which is difficult to detect when many univalents are present. Usually division of the univalents occurs at anaphase but their chromatids do not pass to the opposite poles in equal numbers. They show a marked inclination to lag on the spindle between the separating groups of autosomes and it is probably this which occasions the variation in number found in different nuclei at second metaphase in the same individual. When the number of univalents is high, some fail to reach the poles and be included in the 2nd spermatocyte nucleus. At 2nd metaphase one or two are frequently found in the

cytoplasm and removed from the spindle, usually surrounded by a clear area suggesting that they form accessory spindles. In this case variation in the individual is brought about by the loss of these chromosomes.

No irregularity of behaviour on the part of the Y has been found. At second metaphase it lies to one side of the plate in the spindle axis

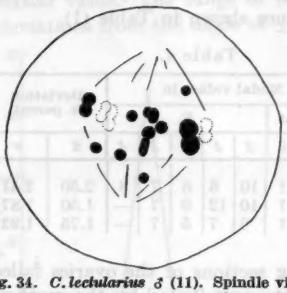

Fig. 34. *C. lectularius* ♂ (11). Spindle view of 2nd metaphase showing univalents which have not reached the plate.

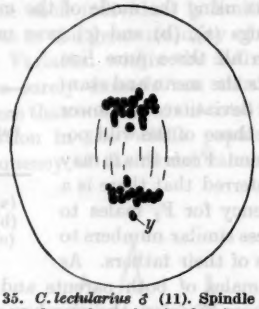

Fig. 35. *C. lectularius* ♂ (11). Spindle view of 2nd anaphase showing lagging of univalents.

and frequently but not invariably in closer apposition to two elements of the central group than to others, though more may lie close together about the point opposite to the Y. At this stage the behaviour of the central group depends in part upon the number of its members. Where this is low, all lie approximately in the equatorial plane in an irregular manner, but increase in number frequently causes a crowding out of several elements which are then distributed along the spindle without showing any positional correlation (Fig. 34). Apparently they fail to reach the equator. Movement of the univalent chromatids at second anaphase resembles that of the first division; they frequently lag behind the autosomes, but appear in all cases to be included finally in the daughter nuclei (Figs. 35, 36).

Fig. 36. *C. lectularius* ♂. 2nd Anaphase showing lagging of univalents on the spindle.

When the number of central elements is low their distribution at second anaphase can be readily ascertained. One specimen was found in which the number was both low and apparently constant since 50 nuclei showed no variation. Second anaphase segregation of the four univalents present in addition to the Y in this specimen, showed that the Y segregated from all the other univalents in the majority of nuclei but also that one or two occasionally accompanied the Y. At least two were always found to pass to the opposite pole.

When dealing with chromosomes as minute as are these of the central group the probable error of measurement must necessarily be very large, in any attempt to evaluate their dimensions. In spite of this fact the elements of the central group do appear to fall into three classes of relative sizes — large, intermediate and small.

In fifteen nuclei, where their number ranged from 4 to 12 per nucleus at second metaphase, there were thirteen cases in which 2 chromosomes belonged to the large class, and two cases having 3 and 6. The small class in the same nuclei varied from 0 to 3, and the intermediate from 2 to 8. The two chromosomes observed to be most frequently associated with the Y were the two of the large class.

The F_1 males, of a pair mating whose male parent was observed to have 2 large, 3 intermediate and 0 to 1 small elements, differed widely from that parent in the relative proportions of the classes. Two large univalents occurred in all F_1 nuclei with one exception which had 6. All numbers from 3 to 8 appeared in the intermediate class while the small class varied from 0 to 3.

Fig. 37. *C. lectularius* ♀. Late diplotene stage in maturation of the ovum.

It would seem from this that the female parent, whose constitution was unknown, contributed at least 2 small and 5 intermediate elements to some of the offspring and at least 4 large to the single exception noted.

Chromosome behaviour in the female cannot be adequately described since only two nuclei showing division have been found. One of these, at late mitotic prophase, gave a count of thirty-eight chromosomes; equivalent to thirteen autosome pairs and twelve univalents. The other showed a late diplotene figure. In this univalents could be distinguished which again amounted to twelve. While most of them were single elements, several seemed to be paired as though their separation was taking place but was still incomplete. The bivalents afforded better evidence of chiasmata than is the case in the male owing to stronger repulsion forces separating the homologues (Fig. 37).

Discussion.

The atypical forms of the bivalents in both *C. rotundatus* and *C. lectularius* indicate the nature of the physical system governing their behaviour. The absence of distinct loops between the points of crossing-over and the fact that the paired homologues remain virtually in one plane appears to be a consequence of a low surface charge producing reduced repulsion forces. Chromosomes with median centromeres do not occur and the stronger repulsion of the centromeres is shown by the wider divergence of proximal than of distal ends.

The type of bivalent with divergent limbs of unequal length is that which is orientated at first metaphase with a long and a short limb

directed towards each of the poles. It is evident that chiasmata are localised in a median segment and are not terminalised, probably because there is a state of equilibrium between the proximal and distal limbs which prevents movement of chiasmata.

The appearance of a transverse split in these bivalents must mean, either that a single chiasma is formed and the chromatids are drawn out to threads too fine to be seen, or that two reciprocal chiasmata regularly occur in the median segment. The latter case would be analogous to the pairing of X and Y chromosomes in *Drosophila* (DARLINGTON 1934). As has been noted repulsion forces in the female appear to be stronger and loops open out between chiasmata. Little can be said of this however since only one late diplotene nucleus has been seen and fixation in that was not perfect. If correct there must be some dimorphism as regards external conditions operating on the chromosomes.

Division of the univalents shows a marked precocity in timing relative to that of the autosomes. It would seem that prophase pairing takes place as in other *Heteroptera*, e. g. in *Corixa* (SLACK 1938a). Probably it is limited to very short terminal segments (DARLINGTON 1937) and is maintained only during diplotene. The cycle of division in the sex chromosomes of *C. rotundatus* and also in the other univalents in *C. lectularius*, is virtually completed at first anaphase without reduction having occurred. Preservation of a mechanism giving a normal sex ratio then depends upon a special property of these chromosomes — the property of secondary attraction and co-orientation at second metaphase. This system is balanced in *C. rotundatus* so that segregation of both X chromosomes from the Y is regular (c. f. STEOPOE 1930, PAYNE 1912), but apparently is not balanced in *C. lectularius*. The question then arises as to how many of the univalents are sex chromosomes essential for the production of a normal sex ratio. Loss of equilibrium in the sex mechanism must entail the death of all ova and sperm having an unbalanced complement. The observations embodied in this paper give no conclusive evidence that the essential sex chromosomes have not the XXY constitution found in *C. rotundatus*. On the contrary, the general occurrence of two univalents distinguished by their size and their freqent close association with the Y supports the idea that the two species are alike in this respect.

With the exception of regular segregation, all the univalents in *C. lectularius* behave like X chromosomes and it would seem that they have some relationship as though they possessed genes which govern the behaviour of sex chromosomes without those genes which determine sex. Otherwise their wide variation in number without visible change in the phenotype or break down of meiosis proclaims them to be genetically inert, and they may be of similar character to the B chromosomes of *Zea mays* (RANDOLPH 1928) which they resemble to some extent in behaviour.

Although unbalanced, a partial equilibrium in *C. lectularius* is apparent from the lack of completely random distribution. Two univalents plus the Y chromosome have not been found in the male, but second anaphase nuclei often contain only the Y, especially when the number of univalents is low. Some must therefore have been contributed by the female parent in addition to a possible two X chromosomes. At the other end of the scale, complete segregation of all elements from the Y would lead to higher numbers than the maximum (16) found if any were brought into the zygote by the female. Given random segregation, the chances of all but the Y going to one pole will become less as the number of univalent elements increases and the absence of higher numbers than 16, in the material examined, might be due to the rarity of higher values.

That random segregation does not exist is shown by considering the case of the female nucleus with 12 univalents. Here, even with equal segregation, the number contributed to the zygote would be 6, which, according to the observed range of variation limits the maximum number supplied by the sperm to 10. Some form of regulation must evidently exist, preserving the limiting values for the number of univalents which can be present. Since one or two univalents are frequently lost at first anaphase in spermatogenesis when their number approaches the maximum, this may be the control required.

As regards the derivation of the variable number of univalents in *C. lectularius*, it is significant that this species lacks one pair of autosomes compared with those of *C. rotundatus*. The missing autosomes may have become inert and have taken on the behaviour of sex chromosomes by progressive changes in their relative timing of division. The commencement of such a change is shown in the behaviour of 'm' chromosomes in *Notonecta*, which also may be unpaired and divide equationally at the first division (Browne 1916). On the other hand it is possible that this pair of autosomes fragmented so that two short fragments, each with a centromere, formed a base for the attachment of segments translocated from the X chromosomes, segments which contained genes controlling behaviour of sex chromosomes but otherwise inert.

In whatever way the autosomes became transformed, hybridisation would build up the present series of univalents.

Summary.

1. Prophase pairing during spermatogenesis in *Cimex* exhibits reduced body repulsions leading to anomalous types of bivalents at metaphase.

2. *Cimex rotundatus* possesses 14 pairs of autosomes and 3 sex chromosomes (X X Y) which are constant in number and regular in their segregation at second anaphase. *C. lectularius* has 13 pairs of autosomes and

a number of univalent chromosomes in addition to the Y. All of these behave like sex chromosomes and the presence of at least two appears to be essential, otherwise their number varies. Their segregation at second anaphase is irregular but not completely random.

3. The number of univalent elements varies both in different individuals and in different nuclei of the same individual.

4. This variation in different individuals is found to have limiting values of 4 and 16; while there is a variation of $\pm$ 3 in second metaphase nuclei of each individual.

5. Reasons are given for supposing that the numbers of univalents in the female are similar to those of the male and that progressive increase in number due to their combination is limited by the loss of some univalents at first anaphyse.

6. The distinct size and frequent secondary pairing of two univalents with the Y indicates that two X chromosomes, essential for determination of sex, are present in all nuclei as in *C. rotundatus*. The other univalents, while behaving like X chromosomes, are thought to be genetically inert and allied to the X chromosomes.

Acknowledgement.

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References to Literature.

Browne, E. N.: A comparative study of six species of *Notonecta*. J. Morph. a. Physiol. **27**, 119 (1916). — **Darlington, C. D.:** Anomalous chromosome pairing in the male *Drosophila pseudo-obscura*. Genetics **19**, 95—118 (1934). — Recent advances in Cytology, 2nd Ed., p. 375. London 1937. — **Gunn, W. C.:** The Bed Bug *(Cimex lectularius)*: Prevention of House Infestation, p. 7. Edinburgh: H. M. Stationery Office 1933. — **Harvey, E. B.:** Chromosome numbers in Metazoa. J. Morph. a. Physiol. **28**, 1—64 (1916). — **Patton, W. S.:** Insects, Ticks, Mites and venomous Animals, Vol. 11, p. 303 (1931). — **Payne, F.:** Some new types of chromosome distribution and their relation to sex. Biol. Bull. Mar. biol. Labor. Wood's Hole **16**, 119 (1909). — A further study of chromosomes of *Reduviidae*. J. Morph. a. Physiol. **23**, 331—357 (1912). — **Péterfi, T.:** Abderhaldens Handbuch der biologischen Arbeitsmethoden, Bd. 5 (2), S. 479—561. 1924. — **Randolph, L. F.:** Chromosome numbers in *Zea mays*. Cornell Univ. Agricult. exper. Stat. Mem. **117**, 3—44 (1928). — **Slack, H. D.:** The Association of Non-homologous Chromosomes in *Corixidae (Hemiptera-Heteroptera)*. Proc. roy. Soc. Edinburgh, **58** (2), 192—212 (1938a). — Chromosome numbers in *Cimex*. Nature (Lond.) **142**, 358 (1938b). — The chromosomes of *Cimex*. Nature (Lond.) **1 3**, 18 (1938c). — **Steopoe, G.:** La spermatogenèse chez *Nepa cinerea*. Ann. Sci. Univ. Jassy **16**, 648 (1930). — **Wilson, E. B.:** Studies on the Chromosomes. V. The chromosomes of *Metapodius*. A contribution to the hypothesis of the genetic continuity of chromosomes. J. of exper. Zool. **6**, 147—206 (1909).

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COMPETITION FOR CHIASMATA IN DIPLOID AND TRISOMIC MAIZE.

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With 5 figures in the text.

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1. Introduction.

It has been shown in the past that the frequencies of chiasma formation, or crossing-over, in different bivalents of the same nucleus may not be independent. Negatively correlated frequencies have been observed by genetical means in *Drosophila* species (MORGAN, BRIDGES and SCHULTZ 1933, STEINBERG 1936, MCKNIGHT 1937) and also found cytologically in a number of organisms (DARLINGTON 1933, MATHER and LAMM 1935, MATHER 1936). The existence of such a negative correlation between the chiasma frequencies of different bivalents indicates that the variance of the total number of chiasmata in a nucleus is less than the sum of the variances of the individual bivalents. There are three ways in which the situation might be achieved, (a) by an upper limit to the number of chiasmata possible in a nucleus, (b) by a lower limit, (c) by both an upper and a lower limit. The cytological observations strongly favour the first possibility. Species with few chiasmata per nucleus show negative correlations less frequently than do others with many chiasmata (MATHER 1936). Further LAMM (1936) has shown that in inbred rye, a reduction of the chiasma frequency is accompanied by a lowering of the correlation. Thus there is no evidence of the existence of a lower limit, whereas all the results are explicable in terms of the existence of an upper limit. The bivalents apparently compete for a limited number of chiasmata, and the process may conveniently be referred to as "competition". It is not to be supposed that the limit is invariable and absolute. It may differ slightly from nucleus to nucleus but if this is the case, its variability must be independent, or largely so, of the agents controlling chiasma formation in the individual bivalents.

The upper limit to the number of chiasmata which can be formed in a nucleus may be effective or ineffective. If the other conditions are such that the bivalents would be able to form more chiasmata, but are restrained by the existence of this upper limit, the this limit is *effective* in determining a negative correlation. It is *ineffective* where the bivalents are incapable of jointly forming sufficient chiasmata to bring the limit into operation.

The actual mechanism underlying the working of the limit is at present a subject for speculation only, but there is some evidence that the

limit itself is under genotypic control. MATHER and LAMM (l. c.) and LAMM (l. c.) have shown that Swedish rye normally exhibits competition of its seven bivalents. Now DARLINGTON (l. c.) finds evidence of competition between these seven bivalents, taken as a whole, and the short eighth bivalent in Japanese eight-chromosome rye. Further, his figures fail to show any competition among the seven long chromosomes. Finally the eight-chromosome rye shows more chiasmata per nucleus than does the Swedish seven-chromosome type. The addition of the extra short bivalent has apparently raised the level of the upper linit to the number of chiasmata per nucleus. Of course, a comparison between the behaviour of a Japanese rye and a Swedish form must be made with caution, and it was to obtain more critical evidence of the relation of the upper limit to the genotype that the present investigation was undertaken.

Zea Mays was chosen as the material for several reasons. Families segregating diploids and various trisomic types were easy to obtain, and sufficiently good preparations of meiosis are not too difficult to make. Furthermore there is but little reduction in the number of visible chiasmata between diplotene and metaphase (DARLINGTON 1934). Hence the latter stage, which is the easier to observe, may be used for the purpose of obtaining chiasma frequencies. A small reduction in the number of chiasmata would presumably "damp" the negative correlation but slightly, and hence is not objectionable. Thus the effect of the addition of an extra chromosome to the normal complement could readily be determined.

The seed was supplied by Dr. M. M. RHOADES and grown for me by Dr. E. G. ANDERSON. Facilities for the cytological examination were provided by Dr. K. SAX, and the work was carried out during my tenure of a Rockefeller International Fellowship. I wish to express my gratitude for this co-operation and assistance.

2. Material.

All the plants analysed were grown in a greenhouse at the California Institute of Technology during the winter 1937/38, fixations of the tassels being made in February and March 1938. The preparations were made according to the instructions of MCCLINTOCK (1929) with the slight modification that they were mounted in euparal directly from absolute alcohol. This saves using a clearing mixture of alcohol and xylol, with its tendency to cloud.

In accumulating data for statistical analysis it is necessary to avoid selection. Unless about 90% of the nuclei, as a minimum, are fully analysable a slide must be discarded; otherwise it would be possible to ascribe some or all of the results to the use of selected data. All the data presented are from slides which passed this test. There were very few borderline slides; as a rule they were clearly good enough, or very poor.

Four different families were grown. The first (No. 1) segregated plants trisomic for the short arm of chromosome V (RHOADES 1936). Nos. 2

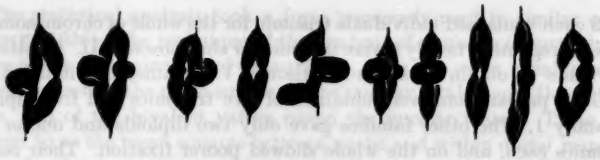

Fig. 1[1]. Diploid from family 1. 10^{II}.

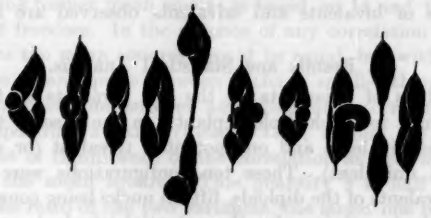

Fig. 2[1]. Diploid from family 2. 10^{II}.

Fig. 3[1]. Trisomic from family 1. $10^{II} + 1^{I}$.

Fig. 4[1]. Trisomic from family 1. $9^{II} + 1^{III}$.

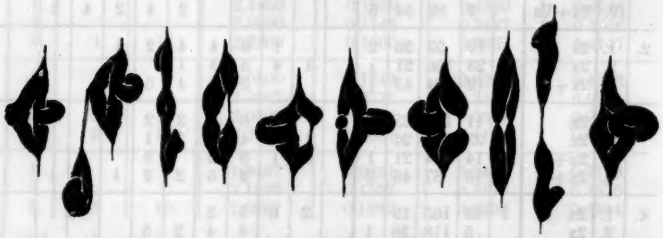

Fig. 5[1]. Trisomic from family 2. $9^{II} + 1^{III}$.

[1] See note at the foot of Table 2.

and 3 each contained individuals trisomic for the whole of chromosome V, while segregants of family 4 were trisomic for chromosome III. An attempt was made to obtain divisions in trisomic VII plants, but it failed.

Good preparations were obtained of five trisomics and five diploids of family 1. The other families gave only two diploids and one or two trisomics each, and on the whole showed poorer fixation. Their results merely serve to corroborate the main data from Family 1.

The types of bivalents and trivalents observed are illustrated in Figs. 1—5.

3. Results and Statistical Analysis.

The numbers of chiasmata in the ten bivalents of fifteen nuclei were determined for each diploid plant. In the case of the trisomics, there are nine bivalents and one potential trivalent (or corresponding bivalent and univalent). These ten configurations were treated just as the ten bivalents of the diploids, fifteen nuclei being counted as before. The ten bivalents, or nine plus the potential trivalent, are not consistently distinguishable at the first meiotic metaphase. It is necessary therefore to base all the analyses on two frequency distributions, viz. the number of chiasmata per bivalent and the number of chiasmata per nucleus. The data for each plant are given in Table 1.

Table 1.

Family and Plant		Type[1]	Frequencies of bivalents with chiasmata					Frequencies of nuclei with chiasmata									
			0	1	2	3	4	17	18	19	20	21	22	23	24	25	26	27	28
1.	1.	2x		19	99	32			1		5	5	2	2					
	2.	2x		17	107	24	2		1	1	4	5	3	1					
	3.	2x		8	112	29	1				2	2	5	2	2	1	1		
	4.	2x		8	95	46	1					3	5	3	2	2			
	5.	2x		18	107	23	2		1	1	4	6	3						
	6.	2x+Vs		15	87	45	3			1		1	8	2	2		1		
	7.	2x+Vs		13	88	48	1				2	2	5	2	2	1	1		
	8.	2x+Vs		6	72	66	6						1	3	2	4	3	1	1
	9.	2x+Vs		13	81	53	3				1	2	3	3	3	1	2		
	10.	2x+Vs		2	89	54	5						2	4	2	4	3		
2.	1.	2x		19	93	36	2			1	3	4	4	2	1				
	2.	2x		23	105	21	1		1	4	5	4	1						
	3.	2x+V		12	94	43	1				2	2	4	5	2				
3.	1.	2x		11	116	23				2	6	2	3	2					
	2.	2x		23	103	24		1	1	4	4	1	3	1					
	3.	2x+V		14	114	21	1		1	1	5	6		2					
	4.	2x+V		15	87	46	2				2	5	2	2	1	2	1		
4.	1.	2x	1	25	105	19			2	6	5	2							
	2.	2x		5	118	26	1				4	4	2	5					
	3.	2x+III		20	112	18		2		3	4	5	1						

[1] Vs = short arm of chromosome five, V = chromosome five, III = chromosome three.

The statistical analysis took a form previously used in similar cases (MATHER 1936). The technique of the analysis of variance (FISHER 1936) allows of the determination of the variance, or as it is more usually called in this connection the mean square, of the nuclear totals round their plant mean, and of the bivalent values round the nuclear means. These are referred to as the mean squares between nuclei and within nuclei respectively. In the present case, where the bivalents are followed in each of 15 nuclei, the former mean square is based on 14 and the latter on 135 degrees of freedom. In the absence of any correlation between the bivalent values the mean squares should be equal, but with a negative correlation the mean square between nuclei is smaller than that within nuclei. A convenient measure of any observable correlation is given by the corresponding z value (FISHER 1936, section 39), which also serves as a test of significance of the correlation as determined by the inequality of the mean squares. This quantity z is half the natural logarithm of the ratio of the two variances, the larger one being divided by the smaller (z is never negative). In the present data the mean square between nuclei was always the smaller and hence always the

Table 2.

Family and Plant		Numbers of nuclei with[1] $10^{II}+1^{I}$	Numbers of nuclei with[1] $9^{II}+1^{III}$	Mean (m)	Mean Square between (V_B)	Mean Square within (V_W)	z $\left(\frac{1}{2}\log_e\left[\frac{V_W}{V_B}\right]\right)$
1.	1.			2.0867	0.1695	0.3519	0.3652
	2.			2.0733	0.1638	0.3400	0.3652
	3.			2.1533	0.0838	0.2689	0.5829
	4.			2.2667	0.1810	0.3319	0.2916
	5.			2.0600	0.1257	0.3459	0.5061
	6.	5	10	2.2400	0.2400	0.4444	0.3081
	7.	4	11	2.2467	0.2681	0.3830	0.1783
	8.	6	9	2.4800	0.2743	0.4267	0.2208
	9.	4	11	2.3067	0.3210	0.4400	0.1577
	10.	10	5	2.4133	0.1981	0.3526	0.2882
2.	1.			2.1400	0.1829	0.4259	0.4226
	2.			2.0000	0.1143	0.3437	0.5506
	3.	1	14	2.2200	0.1600	0.3667	0.4147
3.	1.			2.0800	0.1743	0.2267	0.1322
	2.			2.0067	0.2781	0.3193	0.0691
	3.	3	12	2.0600	0.1686	0.2674	0.2304
	4.	2	13	2.2333	0.3667	0.4126	0.0589
4.	1.			1.9467	0.0831	0.3437	0.7129
	2.			2.1533	0.1552	0.2170	0.1676
	3.	5	10	1.9867	0.1982	0.2593	0.1344

[1] $10^{II}+1^{I}=10$ bivalents and 1 univalent. $9^{II}+1^{III}=9$ bivalents and 1 trivalent.

For degrees of freedom 135 against 14. — 5% point of $z=0.3834$. — 1% $=0.5503$.

denominator. Where V_B and V_W are the mean squares between and within nuclei respectively

$$z = \frac{1}{2} log_e \left[\frac{V_W}{V_B}\right]$$

The mean chiasma frequency, the mean squares between and within nuclei and the z value are given for each plant in Table 2 (see page 123). As noted above, there is at least some suggestion of a negative correlation in each of the twenty plants. The significance of the z value is easily tested. With 135 degrees of freedom for the larger and 14 for the smaller variance z will equal or exceed 0.3834 in 5% of cases by chance, in the absence of correlation. The corresponding 1% point is 0.5503. With either level of significance some plants show evidence of real correlations while others do not.

Our prime purpose is however not concerned with the values of any individual plants but is to determine whether there exists a difference between the diploids on the one hand and their sister trisomics on the other. Considering the plants of family 1, the five diploids have a mean z value of 0.42220, while their five trisomic sisters give $\bar{z} = 0.23062$. The most stringent test of significance which can be applied to the difference of these two $\bar{z}$'s is to treat the five z values of each class as an observed distribution and to calculate a t from the difference between the two means and its estimated standard error (FISHER 1936, section 24. 1). Table 3 shows the working for this test.

Table 3.

	2x	2x + Vs
$S(z)$	2.1110	1.1531
$\frac{S(z)}{5} = \bar{z}$	0.42220	0.23062
$S(z - \bar{z})^2$	0.05642	0.01547
$\frac{S(z-\bar{z})^2}{4} = V_z$	0.01411	0.00387
$\frac{V_z}{5} = V_{\bar{z}}$	0.002821	0.000774
d_z	0.1916	
V_d	0.003595	
$\sqrt{V_d} = s_d$	0.05996	
$\frac{d_z}{s_d} = t_z$	3.195	
Degrees of Freedom	8	
Probability	0.02—0.01	

Table 4.

	2x	2x + Vs
$S(m)$	10.6400	11.6867
$\frac{S(m)}{5} = \bar{m}$	2.12800	2.33734
$S(m - \bar{m})^2$	0.02920	0.04475
$\frac{S(m-\bar{m})^2}{4} = V_m$	0.00730	0.01119
$\frac{V_m}{5} = V_{\bar{m}}$	0.001460	0.002238
d_m	0.2093	
V_d	0.003698	
$\sqrt{V_d} = s_d$	0.06081	
$\frac{d_m}{s_d} = t_m$	3.442	
Degrees of Freedom	8	
Probability	< 0.01	

The difference between the means (d) is 0.42220—0.23062 = 0.1916. The variance of this difference (V_d) being the sum of the two V_z values = 0.002821 + 0.000774 = 0.003595.

$$\text{Then } t = \frac{d}{s_d} = \frac{d}{\sqrt{V_d}} = \frac{0 \cdot 1916}{0 \cdot 05996} = 3.195.$$

This t value has eight degrees of freedom, 4 from each distribution (as each contains five z values). On entering in FISHER's table of t we find that for eight degrees of freedom t will equal or exceed this value of 3.195 by chance less than once in fifty trials, in the absence of a real difference. This is good evidence for believing that there exists a real discrepancy between the means of the diploid and trisomic z's. The trisomics show a lower negative correlation than do their diploid sisters. We may also note that the z values of both classes differ significantly from 0, the expectation with no correlation.

Certain features of the t test are worthy of emphasis. It is an empirical test based solely on the z values actually observed. Any general effect of terminalisation, chromosome size difference etc. in raising or lowering the correlations observed, and any agents increasing differences between plants of the same kind, even if of importance, can affect the t only by lowering its value, i. e. by decreasing the significance of the difference in which we are interested. Where the t has a significant value every confidence may be placed in the reality of the difference between the means.

We may apply the same technique to consideration of the mean numbers of chiasmata per bivalent in the diploid and trisomic plants of family 1. The mean of the five diploid means is 2.12800 and that of the five trisomic means 2.33734. The difference is 0.20934. The variance of this difference, found in Table 4, is 0.003698 and

$$t = \frac{0.20934}{\sqrt{0.003698}} = 3.442 \text{ for 8 degrees of}$$

freedom as before. The probability of this t value is rather less than 1 per cent, so we may judge the trisomics to form more chiasmata than the diploids.

The data from families 2, 3 and 4 are scantier.

In family 2 the single trisomic has a higher mean and lower correlation than either of its diploid sibs. Though the differences are not great, they agree in direction with the results from family 1.

The two diploids of Family 3 agree well with each other in both their z values and their means. Their z values are small, and we may expect this low correlation to be accompanied by a different diploid-trisomic comparison from that observed in family 1. This expectation is fulfilled in that neither trisomic has both a higher mean and lower

z than the diploids. There is no ground for supposing that these trisomics are unlike their diploid sisters, as they deviate in opposite ways.

Family 4 requires little comment. With the exception of a strangely high z value in one diploid, all three plants agree well in their means and correlation. As in family 3, this agreement is associated with low correlation, always excepting the one peculiar diploid. The behaviour of this plant is anomalous and probably of little significance for the present analysis.

4. Discussion.

The outstanding features of the results given above are the differences in mean and correlation between the diploids and trisomics of family 1. The second family probably shows the same difference, the remaining two fail to do so, though in all these three cases the data are meagre and hence unreliable.

In order to appreciate the full significance of the present results it will be necessary to recall certain features in the cytological behaviour of trisomics. Where a chromosome is represented twice, as in a diploid, the two homologues are fully associated at the pachytene stage of the first meiotic division. With four homologues, two pairs are formed, though exchanges of partner may occur. In the case of three homologues in the same nucleus, the situation is somewhat different, as only two of them are paired at any level, the third being left unpaired. Thus, though occasional changes of partner bring all three into association, the length available for chiasma formation is just the same as in the disomic condition. If then the addition of an extra chromosome to a diploid raises the effective upper limit of the number of potential chiasmata, it should produce an increase in the mean number of chiasmata per configuration and a decrease in the negative correlation. This is so because there is still the same length of paired chromosome available for chiasma formation and the increased mean can only be achieved by an increased number in those configurations whose chiasma formation was previously hindered by that of the other bivalents. These may now form chiasmata more freely in the presence of a less restraining upper limit. This curious expectation is fully realised in practice, and we may thus have every confidence that the addition of an extra short arm of chromosome V does raise the upper limit. The limit is in fact under genotypic control.

We may note also that in the event of the upper limit being ineffective in the diploid, the extra chromosome should have no effect at all, as the situation with regard to its operation is unchanged. This is presumably the case in families 3 and 4, possibly as a result of an intrinsically lower chiasma frequency.

It is conceivable that the change in genotype might sometimes lower the effective limit. In this case there will be a decreased mean

with or without an increase in the negative correlation. This has not yet been observed in maize or elsewhere.

We may carry the analysis still further in the case of family 1, though with less precision. The raised upper limit should lead to an increase in the number of chiasmata formed by each and all of the competing configurations. Thus the addition of an extra piece of chromosome V should lead to the formation of more chiasmata in all ten chromosomes, the trivalent being the same as a bivalent for this purpose. Now in the 75 nuclei, 15 from each of the five diploids in family 1, a total of 1596 chiasmata were found. In the corresponding 75 trisomic nuclei 1753 chiasmata were observed. Thus the trisomic nuclei have 157 chiasmata in excess of the diploids. How many of the 157 may be accounted for by increased chiasma formation in Chromosome V, the trisomic configuration? This can be answered approximately.

Of the 75 potential trivalents, one in each nucleus, 46 were observed to form actual trivalents; the other 29 were present as bivalents and univalents. Now the 46 trivalents (which could, of course, be distinguished from the bivalents of the same nucleus) contained 115 chiasmata in all. If the remaining 29 cases formed chiasmata at the same rate, the 75 potential trivalents would contain $\frac{115 \times 75}{46}$ or 187.5 chiasmata. Now the diploid had 1596 chiasmata in all, or 159.6 chiasmata per bivalent, so chromosome V had an excess of 187.5—159.6 or 27.9 chiasmata in the trisomic, as compared with its behaviour in the diploid. This is but a small portion of the total excess of 157 chiasmata, so some or all of the other chromosomes must also have crossed over at a higher rate.

There are two obvious overestimates in this calculation. In the first place it may be wrong to suppose that the 29 potential trivalents which were actually found to be represented as bivalents and univalents have the same chiasma frequency as the 46 observed associations of three. From Table 2 it will be seen that there is no clear correlation between trivalent formation and chiasma frequency when different plants are compared, though of course this does not necessarily mean that there will be no such correlation within plants. In any case if such a correlation exists it should be positive, i. e. the trivalents actually formed should have a higher mean chiasma frequency than those failing to form, since metaphase association is by chiasmata. Thus in basing the estimate of the frequency of all 75 potential trivalents on the 46 observed we should be making an excessively high estimate. This error would result in underestimation of those excess chiasmata in the trisomics, which must be ascribed to the other nine chromosomes, and so react adversely to our argument. The demonstration of the formation

of more chiasmata by the other nine chromosomes in the trisomic is thus still good.

The second error arises from the assumption that chromosome V in the diploid forms $^1/_{10}$ of all the chiasmata of such plants. Now chromosome V is slightly longer than the mean of all ten (RHOADES and MCCLINTOCK 1933) and so might be expected to form rather more than the average number of 159.6 assigned to it. The error would again give a spuriously low value to the number of extra chiasmata ascribed to the other nine chromosomes of the trisomics and the argument still stands.

In general the overestimates made would tend to obscure the effect which is being sought. Then, as such an effect is still observable we can feel reasonably confident that the raising of the effective upper limit by the extra piece of chromosome V has in fact led to an increased number of chiasmata in chromosomes other than V. In addition to any local effect on chromosome V, the extra chromosome has an influence on the nucleus as a whole, as would be expected on the competition hypothesis.

The raising of the upper limit by the addition of extra chromatin is possibly the explanation of the phenomenon, noted by DARLINGTON and MATHER (1932), that triploids have higher chiasma frequencies than the corresponding diploids. The explanation advanced by these authors depended on intra-chromosome interference, but the new possibility seems in some ways more likely. In general if the diploid shows competition, the corresponding triploid should have a higher mean chiasma frequency per configuration. But in the tetraploid, with complete pachytene pairing, the number of chiasmata per bivalent or potential bivalent may be reduced below the diploid value, as the increase in the upper limit may not be sufficient to compensate for the doubling of the length of paired chromosome available for chiasma formation. This appears to be the case in *Tulipa* (UPCOTT 1939), though the evidence for the existence of competition in the diploids is not good. In the absence of an effective upper limit, the frequency of chiasma formation per bivalent in the diploid, per trivalent in the triploid and per potential bivalent in the tetraploid should be constant, as has been observed in the tomato (UPCOTT 1935).

In conclusion it should be emphasised that, beyond showing genotypic control of the upper limit, the present observations cast no light on the mechanism of competition. The behaviour of plants carrying various extra whole chromosomes and also fragments of chromosomes may be helpful. Maize should be useful material for this purpose, particularly as it has B chromosomes whose effects may be most enlightening.

Summary.

The addition of an extra chromosome, in particular the short arm of chromosome V, in *Zea Mays* is shown to lead to (a) less competition

for chiasmata between the chromosomes, (b) an increase in the number of chiasmata formed. These effects are in accordance with the hypothesis of control by an effective upper limit to the number of chiasmata which may be formed in any nucleus. The addition of an extra chromosome raises the upper limit of the number of possible chiasmata. This is further supported by the fact that the increase in chiasma formation is not confined to the trisomic chromosome but is, as far as can be judged, shared by all the bivalents. The bearing of these findings on chiasma frequency studies of polyploid series is briefly discussed.

References.

Darlington, C. D.: The origin and behaviour of chiasmata, VIII. *Secale cereale* (n, 8). Cytologia **4**, 444—452 (1933). — The origin and behaviour of chiasmata, VII. *Zea Mays*. Z. Abstammgslehre **67**, 96—114 (1934). — **Darlington, C. D.** and **K. Mather:** The origin and behaviour of chiasmata, III. Triploid *Tulipa*. Cytologia **4**, 1—15 (1932). — **Fisher, R. A.:** Statistical Methods for Research Workers. 6th Ed. Edinburgh 1936. — **Lamm, R.:** Cytological studies of inbred rye. Hereditas (Lund.) **22**, 217—240 (1936). — **Mather, K.:** Competition between bivalents during chiasma formation. Proc. roy. Soc. Lond. B **120**, 208—227 (1936). — **Mather, K.** and **R. Lamm:** The negative correlation of chiasma frequencies. Hereditas (Lund.) **20**, 65—70 (1935). — **McClintock, B.:** A method for making aceto-carmin smears permanent. Stain-Technol. **4**, 53—56 (1929). — **McKnight, R. H.:** Crossing-over in the sex chromosome of racial hybrids of *Drosophila pseudoobscura*. Genetics **22**, 249—256 (1937). — **Morgan, T. H., C. B. Bridges** and **J. Schultz:** Constitution of the germinal material in relation to heredity. Carnegie Inst. Year Book **32**, 298—302 (1933). — **Rhoades, M. M.:** A cytogenetic study of a chromosome fragment in maize. Genetics **21**, 491—502 (1936). — **Rhoades, M. M.** and **B. McClintock:** The cytogenetics of maize. Bot. Rev. **1**, 292—325 (1935). — **Steinberg, A. G.:** The effect of autosomal inversions on crossing-over in the X-chromosome of *Drosophila melanogaster*. Genetics **21**, 615—624 (1936). — **Upcott, M.:** The cytology of triploid and tetraploid *Lycopersicum esculentum*. J. Genet. **31**, 1—20 (1935). — Structural hybridity in Tulipa III. Meiosis in polyploids. J. Genet. **37**, 303—340 (1939).

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GESCHLECHTSUMWANDLUNG BEI SPHAEROCARPUS DURCH VERLUST EINES STÜCKES DES X-CHROMOSOMS.

Von

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Mit 1 Textabbildung und 24 Abbildungen auf Tafel 1.

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1. Einleitung und Problemstellung.

Sphaerocarpus Donnellii ist ein streng zweihäusiges Lebermoos. Die weiblichen, d. h. die archegonientragenden, und die männlichen, d. h. die antheridientragenden Pflanzen sind auch im Habitus stark voneinander verschieden. Es ist schon lange bekannt, daß für den Unterschied der weiblichen und der männlichen Pflanzen ein Unterschied der genetischen Konstitution verantwortlich ist, und zwar ein einfach mendelnder Unterschied. Da bei *Sphaerocarpus Donnellii* in den Sporogonen jeweils die 4 durch die Reduktionsteilung aus einer Sporenmutterzelle hervorgehenden Sporen zu einer Sporentetrade vereinigt bleiben, können wir leicht, durch die Untersuchung der Eigenschaften der 4 jeweils aus einer Sporentetrade hervorgehenden Pflanzen, die genetische Tetradenanalyse durchführen. Dabei ergibt sich, daß 2 von diesen 4 Pflanzen immer weiblich und 2 immer männlich sind, und dies ist eben der klarste Beweis dafür, daß es sich um einen einfachen, mendelnden Unterschied handelt, der den Unterschied der beiden Geschlechter bedingt.

Die weiblichen und die männlichen Pflanzen unterscheiden sich auch in ihren Chromosomensätzen (Allen, Lorbeer u. a.): Der weibliche Chromosomensatz ist $8 = 7 + X$, der männliche $8 = 7 + Y$. Das X- und das Y-Chromosom sind heterochromatisch, die 7 Autosomen euchromatisch. Das X-Chromosom ist das größte Chromosom des ganzen Satzes und hat etwa die 16fache Größe des punktförmigen Y-Chromosoms. Es ist ganz klar, daß der genetische Unterschied zwischen beiden Geschlechtern seine cytologische Grundlage in einem Unterschied zwischen dem X- und dem Y-Chromosom haben muß. Deshalb ist es berechtigt, diese Chromosomen als Geschlechtschromosomen zu bezeichnen. Es wäre aber falsch, aus den bisher dargelegten Befunden etwa zu folgern, die in den Autosomen gegebene genetische Substanz habe keine Bedeutung für die Art der geschlechtlichen Ausprägung der Pflanzen, oder überhaupt irgend etwas weiteres aussagen zu wollen über die Beteiligung der verschiedenen Komponenten der genetischen Konstitution an der Ausbildung der mit dem Geschlecht zusammenhängenden Eigenschaften. Wir können auf Grund eines Kreuzungsexperimentes ja nur Aussagen machen über *Unterschiede* in der genetischen Konstitution der Kreuzungspartner, aber nicht über die Wirkung genetischer Faktoren, die bei beiden

Partnern gleich sind. Der genetische Unterschied zwischen den weiblichen und den männlichen Pflanzen allerdings kann allein in den Geschlechtschromosomen liegen.

Nun konnte ich (KNAPP) früher zeigen (2, 3), daß nach Behandlung von Sporenmutterzellen oder Sporen mit Röntgenstrahlen aus ursprünglich weiblich bestimmten Sporen zu einem bestimmten Prozentsatz männliche Pflanzen hervorgehen. Ich erhielt in diesen Fällen aus einer Tetrade also nicht 2 Weibchen und 2 Männchen, sondern 1 Weibchen und 3 Männchen oder auch 0 Weibchen und 4 Männchen. Unabhängig davon und fast gleichzeitig gelang es LORBEER, aus röntgenbestrahlten weiblichen Pflanzen rein männliche Regenerat-Thallusäste zu erzielen (4). Es ist ganz klar, daß in diesen Fällen eine Änderung der genetischen Konstitution, also eine Mutation im weitesten Sinne des Wortes vorgelegen haben muß, die diesen Geschlechtsumschlag bewirkte. Es war aber die Frage zu lösen, welcher Art diese Änderung der genetischen Konstitution war.

Die cytologische Untersuchung der „Umwandlungsmännchen" ergab, daß auch sie 8 Chromosomen besaßen, aber nicht den normalen männlichen Chromosomensatz 7 + Y, sondern 7 + X Chromosomen wie die Weibchen. Wir bezeichnen deshalb diese „Umwandlungsmännchen" auch als „X-Männchen". Von den 4 Pflanzen einer Tetrade besaßen also auch hier immer 2 Pflanzen 7 + X, 2 Pflanzen 7 + Y Chromosomen, nur daß in diesen Fällen 1 oder 2 der X-Chromosomenpflanzen Männchen waren. Nun war mir aber damals bei der cytologischen Prüfung einiger solcher X-Männchen aufgefallen, daß ihr X-Chromosom nicht vollständig war, sondern daß ein Stück fehlte. Ich äußerte deshalb die Vermutung, daß die Geschlechtsumwandlung auf den durch die Bestrahlung verursachten Verlust eines Stückes des X-Chromosoms zurückzuführen sei. Da ich auch bei gleich behandelten weiblich gebliebenen Schwesterpflanzen gelegentlich den Verlust eines Stückes des X-Chromosoms beobachtete, und zwar manchmal den Verlust eines größeren Stückes als das bei den X-Männchen fehlende, zog ich den weiteren Schluß, daß nicht der Verlust eines *beliebigen* Stückes des X-Chromosoms von bestimmter Länge den Geschlechtsumschlag bedingt, sondern nur der Verlust eines *bestimmten* Stückes, das offenbar ein bestimmtes Gen oder eine bestimmte Gruppe von Genen trägt, die für die Ausbildung einer weiblichen Pflanze notwendig sind. Es könnten dann also nicht etwa gleichmäßig über das X-Chromosom geschlechtsbestimmende Gene verteilt sein.

Diese Befunde sind, falls sie zutreffen, aus zwei Gründen von Interesse. Einmal geben sie uns einen Ansatzpunkt zur weiteren Analyse des für die Entscheidung „Weibchen oder Männchen" verantwortlichen genetischen Unterschiedes, also zur Bearbeitung des genetischen Mechanismus der Geschlechtsbestimmung, zum andern würden sie uns zeigen, daß die verlorengegangenen Stücke des X-Chromosoms für die Lebensfähigkeit der haploiden Pflanze nicht notwendig sind, ein Ergebnis, das deshalb von Bedeutung ist, weil im allgemeinen das Vorhandensein

mindestens *eines* vollständigen Chromosomensatzes die Voraussetzung für die Lebensfähigkeit eines Organismus ist.

Nun hat LORBEER in einer kürzlich erschienenen Arbeit meine Befunde bestritten (5). Er kommt auf Grund seiner Untersuchungen zu der zweiten a priori möglichen Auffassung, nämlich der, daß die Geschlechtsumwandlung *nicht* durch den *Verlust* eines Chromosomenstückes, also durch eine Chromosomenmutation, bedingt sei, sondern durch eine Genmutation, und zwar durch die Umwandlung eines im X-Chromosom gelegenen weiblichen Realisators in einen männlichen. Pflanzen, die ein Stück des X-Chromosoms verlieren, seien überhaupt nicht lebensfähig. Wenn ich glaubte, den Verlust eines Stückes des X-Chromosoms festgestellt zu haben, so sei dieses dem X-Chromosom fehlende Stück tatsächlich nicht verlorengegangen, sondern an eines der 7 Autosomen transloziert worden.

Als die genannte Arbeit von LORBEER erschien, hatten wir bereits eine Untersuchung an größerem Material im Gang, die in erster Linie feststellen sollte, ob meine frühere Aussage, daß der bei den cytologischen Stichproben beobachtete Verlust eines Stückes des X-Chromosoms die Ursache der Geschlechtsumwandlung sei, zutraf. Zunächst hat es sich ja dabei nur um eine Vermutung gehandelt, und die Möglichkeit, daß nur zufällig gleichzeitig mit einer durch andere Ursachen bedingten Geschlechtsumwandlung die in den wenigen Stichproben cytologisch festgestellten Stückverluste des X-Chromosoms stattgefunden hatten, war nicht von der Hand zu weisen. Da LORBEER auf Grund seiner Untersuchungen fest behauptet, daß Pflanzen, denen ein Stück des X-Chromosoms fehlt, überhaupt nicht lebensfähig seien und da er deshalb meine cytologischen Befunde anzweifelt, mußte das Material zunächst auch noch in dieser Richtung ausgewertet werden, ehe die Frage nach einer kausalen Beziehung zwischen dem Verlust eines Stückes des X-Chromosoms und der Geschlechtsumwandlung angegriffen werden konnte.

Es soll also im folgenden der Beweis dafür geliefert werden, daß 1. tatsächlich *Sphaerocarpus*-Pflanzen, in denen ein Stück des X-Chromosoms fehlt, *lebensfähig* sind, und daß 2. tatsächlich Chromosomenmutationen, und zwar Verkürzungen des X-Chromosoms, die *Ursache für Geschlechtsumwandlungen* von weiblich nach männlich sind.

2. Material und Methodik.

Am 12. 8. 36 wurde die Kreuzung 1096 zwischen dem ♀ Klon Al ♀ A und dem ♂ Klon 307 A 2c durchgeführt. Der Klon Al♀A stammt direkt, der Klon 307 A 2c in 2. Generation aus Sporenmaterial, das uns 1932 liebenswürdigerweise von Herrn Professor Dr. C. E. ALLEN, Madison, USA., zur Verfügung gestellt worden war. Es standen 17 Töpfe des genannten ♀ Klones für die Kreuzung zur Verfügung, auf denen sich also die Sporogone als Kreuzungsprodukt entwickelten. Die 17 Töpfe wurden bezeichnet als 1096 ① bis 1096 ⑰. ⑭ und ⑰ dienten als

unbestrahlte Kontrollen, die übrigen wurden vom 29. 8. bis 2. 9. 36 in der Reihenfolge ihrer Nummern mit $^1/_2$—1tägigem Abstand bestrahlt, und zwar alle mit Röntgenstrahlen gleicher Qualität und gleicher Dosis (50 KV eff., 4 MA, 15 cm Abstand, 1 mm Al, 37 Min. 20 Sec., etwa 3200 r). Gleichzeitig mit den Bestrahlungen vorgenommene Kontrollfixierungen ergaben, daß während der Bestrahlungen die Meiose ablief. (Extreme Stadien während der Bestrahlungen: Frühe Prophase der 1. Teilung und abgeschlossene 2. Teilung, aber noch ohne Plasmaspaltung.)

Von jedem der 17 Töpfe wurden in der Zeit vom 28. 4. 37 bis 13. 10. 37 die Tetraden aus je 4 Sporogonen auf Benecke-Agar in Petri-Schalen zur Keimung ausgelegt. Wie schon früher beschrieben (2) wurden die Keimpflanzen einzeln auf Agar pikiert und später auf Gartenerde umgesetzt. Insgesamt wurden aus diesen Sporogonen 12404 Pflanzen aufgezogen. Hiervon waren 5881 weiblich, 6523 männlich. Sämtliche männliche Pflanzen, soweit sie nicht aus Tetraden hervorgegangen waren, die außerdem 2 weibliche Pflanzen geliefert hatten — diese Männchen mußten ja ein Y-Chromosom besitzen —, wurden cytologisch daraufhin geprüft, ob sie ein X-Chromosom bzw. ein Bruchstück des X-Chromosoms besaßen oder nicht. Wurde kein X-Chromosom oder X-Chromosomenstück nachgewiesen, so galten sie als normale Männchen. Von den Sporogonen ① A, ⑰ A und ⑤ B wurden sämtliche Männchen, also auch die aus Tetraden mit außerdem 2 Weibchen hervorgegangenen, cytologisch geprüft, wobei sich bestätigte, daß die letzteren nie ein X-Chromosom (oder X-Chromosomenstück) besaßen. Insgesamt wurden etwa 4280 männliche Pflanzen auf das Vorhandensein eines X-Chromosoms bzw. X-Chromosomenstückes geprüft.

Zu dieser Prüfung genügte im allgemeinen die Untersuchung von Interphasekernen im jungen Thallusgewebe. Da das X-Chromosom heterochromatisch ist, läßt sich fast stets eindeutig entscheiden, ob ein X-Chromosom bzw. ein Stück des X-Chromosoms vorhanden ist oder nicht. Nur in Fällen, wo das X-Chromosom sehr stark (auf mehr als $^1/_4$ oder $^1/_8$ seiner normalen Größe) diminuiert ist, kann die Entscheidung schwierig werden, ob ein normales Y-Chromosom oder ein X-Chromosomenstück vorliegt. Dann müssen günstige Prophasen zu Rate gezogen werden. Doch sind diese Fälle so selten, daß sie bei einer Untersuchung über die Häufigkeit der „X-Männchen" nicht ins Gewicht fallen.

Einige wenige dieser X-Männchen wurden einer eingehenderen cytologischen Analyse unterworfen. Vor allem wurden Prophasestadien untersucht, in denen sich Heterochromatin und Euchromatin und damit X-Chromosom und Autosomen leicht unterscheiden lassen. Zur Ergänzung wurden auch Metaphasen und Anaphasen herangezogen. An diesem Material wurde die oben angeschnittene Frage, ob in den Kernen lebensfähiger Pflanzen Stücke des X-Chromosoms völlig fehlen können, behandelt.

Die Frage, ob eine kausale Beziehung besteht zwischen dem Verlust eines Stückes des X-Chromosoms und der beobachteten Geschlechtsumwandlung, erforderte eine statistische Behandlung. Wir verfuhren folgendermaßen: Unter den aus Sporen aus einer Anzahl von bestrahlten Sporogonen der obengenannten Versuchsserie hervorgegangenen Pflanzen wählten wir 70 Männchen aus, für die die cytologische Voruntersuchung ergeben hatte, daß sie mindestens ein Stück des X-Chromosoms enthielten, die also „Umwandlungsmännchen" waren. Zum Vergleich zogen wir die gleiche Anzahl weiblicher Pflanzen heran, die aus Sporen aus denselben Sporogonen hervorgegangen waren. Um alle eventuell möglichen anderen Ursachen für Unterschiede in der Häufigkeit von Chromosomenmutationen bei den untersuchten Weibchen und den Umwandlungsmännchen auszuschließen, wurde zu jedem Umwandlungsmännchen ein „Vergleichsweibchen" gewählt, das aus einer Spore aus demselben Sporogon hervorgegangen war, aus dem auch das Umwandlungsmännchen stammte. Außerdem stammten Umwandlungsmännchen und Vergleichsweibchen zwar aus verschiedenen Sporentetraden, aber beide aus Tetraden, die bei der Keimung die gleiche Anzahl von Pflanzen insgesamt ergeben hatten. Für die Umwandlungsmännchen und Vergleichsweibchen wurde nun festgestellt, ob und wie häufig sich ein Fehlen eines Stückes des X-Chromosoms nachweisen ließ.

Die Thallusstücke wurden durchweg mit Alkohol-Eisessig fixiert und mit Carminessigsäure mit Zusatz von Eisenchlorid gefärbt. Die zerzupften und gequetschten Vegetationspunkte wurden nach bekannter Methode auf dem Objektträger in Carminessigsäure, zum Teil auch in 45%iger Essigsäure, leicht erhitzt oder aufgekocht. Dieses Verfahren genügte für unsere Zwecke vollkommen und wurde seiner Einfachheit halber beibehalten. Die Präparate wurden zunächst in Kanadabalsam, später in dem alkohollöslichen Euparal (Grübler & Co.) eingedeckt, unter Verwendung der üblichen Zwischenstufen. Die auf Tafel 1 zusammengestellten Mikrophotographien 2—24 sind sämtlich nach so hergestellten Präparaten gemacht, die Mikrophotographie 1 nach einem Präparat direkt in Carminessigsäure.

3. Ergebnisse.

Wenn die Chromosomen von *Sphaerocarpus* sich in ihrem färberischen Verhalten nicht unterscheiden würden, wäre es mit den angewandten Methoden tatsächlich wohl kaum möglich gewesen, zu entscheiden, ob ein am X-Chromosom fehlendes Stück völlig fehlt oder ob es an ein anderes Chromosom transloziert ist. Da aber das X-Chromosom von *Sphaerocarpus* nach allen vorliegenden Untersuchungen total heterochromatisch und die Autosomen euchromatisch sind[1], läßt sich durch Untersuchung der Stadien, in denen sich das Heterochromatin vom Euchromatin färberisch deutlich unterscheidet, also der Interphase und der Prophase, im allgemeinen leicht feststellen, ob ein dem X-Chromosom fehlendes Stück völlig fehlt oder ob es an ein Autosom transloziert ist.

[1] Lediglich einzelne Endchromomeren der Autosomen scheinen mir ebenfalls heterochromatisch zu sein, doch soll auf diese in unserem Zusammenhang unwesentliche Frage hier nicht eingegangen werden.

Schon die Massenuntersuchung der Männchen zur Prüfung, ob sie ein X-Chromosom bzw. X-Chromosomenstück besitzen oder nicht, ergab meines Erachtens eindeutige Befunde dahingehend, daß in manchen Pflanzen die Menge des Heterochromatins gegenüber den normalen Weibchen bedeutend verringert war, und bei Besprechung der Methodik haben wir schon darauf hingewiesen, daß es in manchen Fällen aus diesem Grunde schon schwierig war zu entscheiden, ob ein X-Chromosomenstück oder ein Y-Chromosom vorlag. Uns ist deshalb schwer verständlich, weshalb LORBEER bei seinen Untersuchungen männlicher Thallusregenerate aus röntgenbestrahlten weiblichen Pflanzen keine Verluste von Stücken des X-Chromosoms gefunden hat. Die eingehendere cytologische Untersuchung einer Reihe weiblicher und männlicher Pflanzen, die wenigstens ein Stück des X-Chromosoms besaßen, ließ in vielen Fällen keinen Zweifel darüber, daß größere oder kleinere Stücke des X-Chromosoms fehlten, ohne sich an anderer Stelle im Kern, etwa an ein Autosom transloziert, zu finden. In Tafel 1 sind, neben Mitosen aus normalen männlichen Pflanzen mit Y-Chromosom (Abb. 1) und aus weiblichen oder männlichen Pflanzen mit X-Chromosom, an dem kein Verlust festzustellen ist (Abb. 2—8), unretuschierte Mikrophotographien von Mitosen aus verschiedenen weiblichen und männlichen Pflanzen unserer Versuchsserie zusammengestellt, bei denen unseres Erachtens am völligen Fehlen von größeren oder kleineren Stücken des X-Chromosoms nicht gezweifelt werden kann (Abb. 9—23).

Voraussetzung für die Beweiskraft der Präparate im genannten Sinne ist, daß vom X-Chromosom abgetrennte und eventuell an euchromatische Autosomen translozierte Stücke ihre heterochromatischen Eigenschaften beibehalten. Daran können wir aber nach unseren Kenntnissen vom Heterochromatin kaum zweifeln und niemand dürfte die Berechtigung dieser Annahme bestreiten (s. auch S. 136).

Ich habe früher schon betont (3) und auch HEITZ hat darauf hingewiesen (1), daß bei *Sphaerocarpus* schon der Vergleich normaler weiblicher Pflanzen mit ihrem großen X-Chromosom und normaler männlicher Pflanzen mit ihrem kleinen Y-Chromosom ergibt, daß mindestens der größte Teil des X-Chromosoms frei sein muß von für den Gametophyten lebensnotwendigen Genen. LORBEER behauptet dagegen, „daß das X-Chromosom von *Sphaerocarpus Donnellii* in allen seinen Teilen lebensnotwendige Gene trägt", fügt jedoch hinzu, daß es sich um Gene handelt, die „allerdings in erster Linie zur Erhaltung des X-Chromosoms selbst benötigt werden." Später schreibt er: „Die Gene im großen X-Chromosom müssen . . . empfindlich aufeinander abgestimmt sein, so daß ein Stückverlust eine letale Wirkung im X-Fragment führenden Kern zur Folge hat." Er stellt sich also offenbar vor, daß zwar die Gesamtheit der vom X-Chromosom ausgehenden genetischen Wirkungen für den Gametophyten nicht lebensnotwendig ist oder doch, soweit sie lebensnotwendig ist, durch das Y-Chromosom ersetzt werden kann, daß aber

die Wegnahme eines Teiles des X-Chromosoms und damit die Wegnahme eines Teiles der vom X-Chromosom ausgehenden Wirkungen zu einer Störung eines notwendigen Gleichgewichtes der Wirkungen und damit zu Letalität führt. Eine solche Annahme, mag sie wahrscheinlich erscheinen oder nicht, läßt sich nicht von vornherein widerlegen. Nur die experimentelle Entfernung eines Stückes des ganzen X-Chromosoms und der Nachweis der Lebensfähigkeit von Gametophyten, die außer den Autosomen nur das Reststück des X-Chromosoms enthalten, kann diese Annahme widerlegen. Diesen Nachweis glauben wir eindeutig erbracht zu haben.

Es ist wohl selbstverständlich, daß wir durch unsere Feststellung, daß Stückverluste des X-Chromosoms vorkommen, die die Lebensfähigkeit der Gametophyten nicht aufheben, keineswegs bestreiten, daß auch Translokationen zwischen X-Chromosom und Autosomen erfolgen können. Solche Translokationen haben wir ebenfalls beobachtet und in Abb. 24 der Tafel 1 ist eine solche wiedergegeben. In unseren Versuchen fanden wir Translokationen allerdings viel seltener als einfache Stückverluste des X-Chromosoms.

Heterochromatin und Euchromatin sind in dem durch die Translokation entstandenen Chromosom in der Prophase deutlich zu unterscheiden. Diese Präparate liefern somit auch einen Beweis dafür, daß die Unterschiede im färberischen Verhalten von X-Chromosom und Autosomen auch bei Translokationen erhalten bleiben, was wir (S. 135) als Voraussetzung für die Beweiskraft unserer Präparate zum Nachweis von Stückverlusten des X-Chromosoms angeführt haben.

Den Vorgang, wie das X-Chromosomenstück verlorengeht, haben wir selbst nicht beobachtet. Man wird aber auch hier annehmen dürfen, daß ein abgetrenntes Chromosomenstück ohne Spindelfaseranheftungsstelle im Laufe der Mitosen nicht mehr in die Tochterkerne einbezogen wird, weil es sich in den Anaphasen nicht nach den Polen bewegt. Es wird ins Plasma gelangen und dort resorbiert werden.

Auf die Folgerungen, die aus dem Nachweis, daß Gametophyten, denen ein Stück des X-Chromosoms fehlt, lebensfähig sind, hinsichtlich der Frage nach der genetischen Bedeutung des Heterochromatins zu ziehen sind, habe ich bereits früher hingewiesen (3).

Nicht bei allen Umwandlungsmännchen ließ sich cytologisch eindeutig das Fehlen eines Stückes des X-Chromosoms nachweisen. Umgekehrt fanden wir, wie bereits erwähnt, auch weibliche Pflanzen, denen ein solches Stück fehlte. Deshalb mußte ein statistischer Vergleich der Häufigkeiten nachweisbarer Stückverluste bei Umwandlungsmännchen und bei normalen Weibchen durchgeführt werden. Oben wurde bereits besprochen, wie wir dazu vorgingen. Doch sei an dieser Stelle nochmals betont, daß es sich bei diesem Vergleich für uns nur darum handelte festzustellen, ob am X-Chromosom ein Stück fehlte oder nicht, ohne im

einzelnen stets sicher nachgewiesen zu haben, daß das dem X-Chromosom fehlende Stück nicht vielleicht transloziert war. Streng genommen handelt es sich also um die Prüfung, ob eine Beziehung zwischen cytologisch nachweisbaren Chromosomenmutationen des X-Chromosoms und den Geschlechtsumwandlungen besteht. Das Ergebnis des Vergleichs ist in Tabelle 1 wiedergegeben.

Von 70 untersuchten X-Männchen war bei 62, das sind $88{,}6 \pm 3{,}8\%$[1] eindeutig eine größere oder kleinere Verkürzung des X-Chromosoms nachweisbar, bei den restlichen 8 Pflanzen konnte eine solche nicht nachgewiesen werden. Von den zum Vergleich herangezogenen 70 Weibchen war eine Verkürzung bei 38 Pflanzen, das sind $54{,}2 \pm 5{,}9\%$ nachzuweisen, bei den restlichen 32 Pflanzen nicht. Der Unterschied zwischen den Häufigkeiten der nachweisbaren Verkürzungen bei Weibchen und Männchen ist durch mehr als seinen dreifach mittleren Fehler gesichert und kann somit als real gelten. Es ist darum der Schluß berechtigt, daß *eine kausale Beziehung zwischen Verkürzung des X-Chromosoms und der Geschlechtsumwandlung bestehen muß*, die nur so gedeutet werden kann, daß die Verkürzung die Ursache der Geschlechtsumwandlung ist.

Weiterhin ist die Frage zu prüfen, ob es lediglich von der *Länge* des fehlenden X-Chromosomenstückes abhängt, ob eine Geschlechtsumwandlung erfolgt, oder ob nur das Fehlen eines *bestimmten Stückes* des X-Chromosoms diesen Geschlechtsumschlag bewirkt. Wir haben geprüft, ob morphologische Unterschiede verschiedener Teile des X-Chromosoms nachweisbar sind, die ermöglichen würden, ein verlorengegangenes Stück näher zu kennzeichnen, doch ist uns dies nicht gelungen[2]. Wir wagen deshalb weder zu entscheiden, von welchem der beiden Arme ein verlorengegangenes Chromosomstück stammt, noch ob das verlorengegangene Stück endständig oder interkalar ist. Daß aber nicht nur die *Länge* des verlorengegangenen Stückes entscheidend dafür sein kann, ob eine Geschlechtsumwandlung erfolgt oder nicht, geht klar daraus hervor, daß sowohl bei weiblich gebliebenen Pflanzen als auch bei Umwandlungsmännchen das verlorengegangene Stück des X-Chromosoms die verschiedenste Größe haben kann. Deshalb ist man wohl berechtigt, zu behaupten, daß *die geschlechtsbestimmende Wirkung nicht gleichmäßig vom ganzen X-Chromosom ausgeht* und wird als wahrscheinlich annehmen dürfen, daß nur der Verlust eines bestimmten Stückes des X-Chromosoms, das ein bestimmtes Gen oder eine bestimmte Gruppe von Genen trägt, die für die Ausbildung einer weiblichen Pflanze notwendig sind, den Geschlechtsumschlag bedingt (vgl. KNAPP 1935). Bei denjenigen Männchen

[1] In dieser Arbeit ist als Fehler stets der einfache mittlere Fehler nach JOHANNSEN angegeben. Wir sind uns der Mängel dieser Fehlerangabe bei empirischen Werten bewußt, doch mag sie als Anhaltspunkt dienen.

[2] In einigen Fällen ließ sich am X-Chromosom deutlich eine sekundäre Einschnürung feststellen (z. B. im X-Chromosom der Pflanze 1096 (15) D 62 b; s. Tafel 1, Abb. 17—20), doch erfordern diese und andere Fragen der Chromosomenmorphologie noch ein eingehendes Studium, ehe Endgültiges darüber ausgesagt werden kann.

Tabelle 1. Cytologischer Befund am X-Chromosom von 70 X-Männchen und 70 Vergleichsweibchen.

Die Ziffern 0—5 in Spalte V bedeuten ein Maß für den Grad der nachgewiesenen Verkürzung.

Pflanze	Geschl.	Cytologischer Befund	V
⑦ C 20b	♂	Keine Verkürzung nachgewiesen	0
2a	♀	Keine Verkürzung nachgewiesen	0
⑧ D 109d	♂	1 Schenkel stark verkürzt	3
1b	♀	1 Schenkel etwas verkürzt	1
134b	♂	Keine Verkürzung nachgewiesen	0
4a	♀	Keine Verkürzung nachgewiesen	0
137a	♂	1 Schenkel deutlich verkürzt	2
5a	♀	Keine Verkürzung nachgewiesen	0
147c	♂	1 Schenkel bis auf Knopf verkürzt	4
6b	♀	Keine Verkürzung nachgewiesen	0
157b	♂	1 Schenkel etwas verkürzt	1
12a	♀	Keine Verkürzung nachgewiesen	0
⑨ C 65c	♂	1 Schenkel stark verkürzt	3
2c	♀	Keine Verkürzung nachgewiesen	0
66a	♂	1 Schenkel deutlich verkürzt	2
3a	♀	1 Schenkel deutlich verkürzt	2
66b	♂	1 Schenkel deutlich verkürzt	2
3b	♀	1 Schenkel etwas verkürzt	1
85a	♂	1 Schenkel deutlich verkürzt. Außerdem Y-Chromosom	2
5a	♀	Keine Verkürzung nachgewiesen	0
107a	♂	1 Schenkel deutlich verkürzt	2
8a	♀	1 Schenkel deutlich verkürzt	2
143a	♂	Beide Schenkel deutlich verkürzt	3
13a	♀	1 Schenkel deutlich verkürzt	2
⑨ D 5d	♂	1 Schenkel deutlich verkürzt	2
6a	♀	1 Schenkel stark verkürzt	3
20b	♂	1 Schenkel deutlich verkürzt	2
25a	♀	Keine Verkürzung nachgewiesen	0
29a	♂	Keine Verkürzung nachgewiesen	0
13a	♀	Keine Verkürzung nachgewiesen	0
48a	♂	1 Schenkel etwas verkürzt	1
12a	♀	1 Schenkel bis auf Knopf verkürzt	4
84c	♂	Beide Schenkel sehr stark verkürzt	5
22a	♀	Keine Verkürzung nachgewiesen	0
91a	♂	Keine Verkürzung nachgewiesen	0
14a	♀	Keine Verkürzung nachgewiesen	0
97a	♂	1 Schenkel stark verkürzt	3
26a	♀	Keine Verkürzung nachgewiesen	0
113a	♂	1 Schenkel bis auf Knof verkürzt	4
30a	♀	Keine Verkürzung nachgewiesen	0
125c	♂	1 Schenkel stark verkürzt; ein Teil transloziert?	3
39a	♀	Keine Verkürzung nachgewiesen	0
169a	♂	1 Schenkel bis auf Knopf verkürzt	4
31a	♀	Keine Verkürzung nachgewiesen	0
⑪ C 83a	♂	1 Schenkel fast völlig fehlend, zweiter Schenkel stark verkürzt. Außerdem Y-Chromosom	5
52a	♀	Keine Verkürzung nachgewiesen	0
105b	♂	1 Schenkel stark verkürzt	3
55a	♀	1 Schenkel stark verkürzt	3
127c	♂	1 Schenkel bis auf Knof verkürzt	4
54a	♀	1 Schenkel etwas verkürzt	1
157b	♂	1 Schenkel deutlich verkürzt	2
79a	♀	1 Schenkel stark verkürzt	3

Tabelle 1 (Fortsetzung).

Pflanze	Geschl.	Cytologischer Befund	V
⑪ C 158c	♂	1 Schenkel stark verkürzt	3
82a	♀	Keine Verkürzung nachgewiesen	0
159a	♂	1 Schenkel stark verkürzt	3
57a	♀	1 Schenkel etwas verkürzt	1
⑪ D 59a	♂	1 Schenkel deutlich verkürzt	2
51a	♀	Beide Schenkel deutlich verkürzt	3
82b	♂	1 Schenkel stark verkürzt	3
52b	♀	1 Schenkel deutlich verkürzt	2
97b	♂	1 Schenkel deutlich verkürzt	2
56b	♀	Keine Verkürzung nachgewiesen	0
116b	♂	1 Schenkel bis auf Knopf verkürzt	4
57b	♀	Keine Verkürzung nachgewiesen	0
152b	♂	1 Schenkel etwas verkürzt	1
58a	♀	1 Schenkel deutlich verkürzt	2
17a	♂	1 Schenkel stark verkürzt	3
62a	♀	Keine Verkürzung nachgewiesen	0
⑬ C 55a	♂	1 Schenkel bis auf Knopf verkürzt. Außerdem wohl Y-Chromosomen	4
52a	♀	1 Schenkel bis auf Knopf verkürzt	4
56a	♂	1 Schenkel bis auf Knopf verkürzt	4
71a	♀	1 Schenkel stark verkürzt	3
8a	♂	1 Schenkel fast bis auf Knopf verkürzt; zweiter Schenkel stark verkürzt	5
74a	♀	1 Schenkel bis auf Knopf verkürzt; transloziertes Stück?	4
160a	♂	Beide Schenkel stark verkürzt	4
44a	♀	1 Schenkel stark verkürzt	3
⑱ D 98a	♂	1 Schenkel deutlich verkürzt	2
52a	♀	1 Schenkel deutlich verkürzt	2
102a	♂	1 Schenkel stark verkürzt	3
56a	♀	1 Schenkel deutlich verkürzt	2
103a	♂	1 Schenkel bis auf Knopf verkürzt	4
61a	♀	1 Schenkel etwas verkürzt	1
43a	♂	1 Schenkel deutlich verkürzt	2
73a	♀	1 Schenkel stark verkürzt	3
⑮ C 39a	♂	1 Schenkel bis auf Knopf verkürzt. Außerdem Y-Chromosomen	4
22a	♀	Keine Verkürzung nachgewiesen	0
68a	♂	Keine Verkürzung nachgewiesen	0
15b	♀	Keine Verkürzung nachgewiesen	0
72a	♂	1 Schenkel deutlich verkürzt	2
33a	♀	1 Schenkel stark verkürzt	3
117a	♂	1 Schenkel deutlich verkürzt	2
36b	♀	Keine Verkürzung nachgewiesen	0
⑮ D 10a	♂	1 Schenkel bis auf Knopf verkürzt	4
5a	♀	1 Schenkel stark verkürzt	3
67b	♂	Sehr kurzes X-Chromosom; Stück an Autosom transloziert	3
4a	♀	Keine Verkürzung nachgewiesen	0
70a	♂	Beide Schenkel sehr stark verkürzt	5
17a	♀	1 Schenkel etwas verkürzt	1
76a	♂	1 Schenkel stark verkürzt	3
26a	♀	Keine Verkürzung nachgewiesen; überzähliges X-Chromosomenstück?	0
89b	♂	1 Schenkel stark verkürzt, zweiter Schenkel wahrscheinlich verkürzt	3

Tabelle 1 (Fortsetzung).

Pflanze	Geschl.	Cytologischer Befund	V
⑮ D 28a	♀	Keine Verkürzung nachgewiesen	0
94b	♂	1 Schenkel deutlich verkürzt	2
38a	♀	1 Schenkel stark verkürzt	3
103a	♂	1 Schenkel etwas verkürzt	1
62a	♀	1 Schenkel deutlich verkürzt	2
103c	♂	Keine Verkürzung nachgewiesen	0
62b	♀	1 Schenkel stark verkürzt; im anderen Schenkel sekundäre Einschnürung	3
112a	♂	1 Schenkel bis auf Knopf verkürzt	4
39c	♀	1 Schenkel deutlich verkürzt	2
114a	♂	1 Schenkel stark verkürzt	3
43a	♀	Keine Verkürzung nachgewiesen	0
132b	♂	1 Schenkel bis auf Knopf verkürzt; zweiter Schenkel stark verkürzt	5
49a	♀	1 Schenkel bis auf Knopf verkürzt	4
137a	♂	1 Schenkel etwas verkürzt	1
29a	♀	Keine Verkürzung nachgewiesen	0
140a	♂	1 Schenkel bis auf Knopf verkürzt	4
51a	♀	1 Schenkel etwas verkürzt	1
188a	♂	Beide Schenkel stark verkürzt; Stück an Autosom transloziert. Außerdem 2 Y-Chromosomen	4
32a	♀	1 Schenkel etwas verkürzt	1
240a	♂	Keine Verkürzung nachgewiesen. Außerdem Y-Chromosom	0
61a	♀	1 Schenkel deutlich verkürzt	2
⑯ C 18a	♂	1 Schenkel deutlich verkürzt	2
9a	♀	1 Schenkel stark verkürzt. Außerdem wahrscheinlich Y-Chromosom	3
27a	♂	1 Schenkel bis auf Knopf verkürzt	4
3a	♀	1 Schenkel stark verkürzt	3
28a	♂	Keine Verkürzung nachgewiesen	0
46a	♀	Keine Verkürzung nachgewiesen	0
51a	♂	1 Schenkel stark verkürzt	3
36a	♀	1 Schenkel etwas verkürzt	1
54a	♂	1 Schenkel stark verkürzt	3
42a	♀	1 Schenkel stark verkürzt	3
58a	♂	1 Schenkel stark verkürzt	3
65a	♀	Keine Verkürzung nachgewiesen	0
75a	♂	1 Schenkel etwas verkürzt	1
17b	♀	Keine Verkürzung nachgewiesen	0
81b	♂	1 Schenkel stark verkürzt	3
39a	♀	Keine Verkürzung nachgewiesen	0
⑯ D 63a	♂	1 Schenkel stark verkürzt	3
51a	♀	1 Schenkel deutlich verkürzt	2

und sicher auch bei einem Teil der Weibchen, die keinen Verlust eines X-Chromosomenstückes erkennen lassen, wird man annehmen dürfen, daß auch sie ein X-Chromosomenstück verloren haben, das aber zu klein ist, um mit den angewandten Methoden in den Mitosen nachweisbar zu sein.

Wir haben die Größe des dem X-Chromosom fehlenden Stückes in den verschiedenen Umwandlungsmännchen und Vergleichsweibchen abzuschätzen versucht und danach die Stückverluste in 5 Klassen eingeordnet (Spalte V in Tabelle 1). Stellt man nun fest, wieviel X-Männchen

und wieviel Vergleichsweibchen jeder dieser Klassen angehören (Tabelle 2) und drückt man die Häufigkeit der X-Männchen in Prozenten der Summe der Anzahl der X-Männchen und Vergleichsweibchen der betreffenden Klassen aus, so bekommt man das in Abb. 1 kurvenmäßig dargestellte Ergebnis: Je größer das verlorengegangene Stück des X-Chromosoms ist, desto größer ist der Prozentsatz der an dieser Klasse beteiligten X-Männchen. Man wird dieses Ergebnis[1] wohl so zu interpretieren haben, daß beim Verlust eines kürzeren Stückes des X-Chromosoms die Wahrscheinlichkeit, daß gerade das für die Ausbildung einer weiblichen Pflanze notwendige Stück des X-Chromosoms verlorengegangen ist, geringer ist als beim Verlust eines größeren Stückes. Zur Ergänzung ist in Abb. 1 auch die Klasse 0, d. h. die Fälle, in denen sich cytologisch kein Stückverlust nachweisen ließ, eingefügt.

Tabelle 2. Die Anzahl der X-Männchen und Vergleichsweibchen, die unter den je 70 untersuchten Pflanzen in den 5 verschiedenen, den Grad der Verkürzung des X-Chromosoms charakterisierenden Klassen und in der Klasse ohne nachweisbare Verkürzung (0) gefunden wurden.

	0	1	2	3	4	5	
♀	32	9	11	14	4	0	70
♂	8	6	16	20	15	5	70
Σ	40	15	27	34	19	5	140

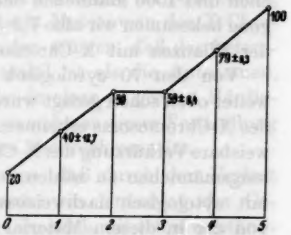

Abb. 1. Die Häufigkeit der X-Männchen in den 5 verschiedenen, den Grad der Verkürzung des X-Chromosoms charakterisierenden Klassen, sowie in der Klasse ohne nachweisbare Verkürzung (0), in Prozenten der in der jeweiligen Klasse gefundenen Gesamtzahl der Pflanzen.

Wenn sich auch, wie bereits betont wurde, die cytologische Untersuchung beim Vergleich der 70 Umwandlungsmännchen mit der gleichen Anzahl von Vergleichsweibchen auf die Frage beschränkte, ob vom X-Chromosom ein Stück abgetrennt ist oder nicht, und die Möglichkeit offengelassen werden muß, daß im einen oder anderen Fall das vom X-Chromosom abgetrennte Stück nicht verlorenging, sondern an ein Autosom transloziert wurde, so kann doch darüber kein Zweifel sein, daß für die nachgewiesene Beziehung zwischen Chromosomenmutationen des X-Chromosoms und Geschlechtsumwandlungen der völlige Verlust eines X-Chromosomenstückes verantwortlich ist. Zu diesem Schluß sind wir deshalb berechtigt, weil wir, wie schon berichtet, in den Fällen, wo wir eine

[1] Grad der statistischen Sicherung des Unterschiedes des Prozentsatzes der untersuchten Pflanzen, die in den verschiedenen „Verkürzungsklassen" auf X-Männchen entfallen: Unterschied zwischen Klasse 1 und Klasse 4: $\frac{\text{Diff}}{m_{\text{Diff}}} = 2{,}48$; Unterschied zwischen Klasse 1 und Klasse 4 + 5: $\frac{\text{Diff}}{m_{\text{Diff}}} = 2{,}93$; Unterschied zwischen Klasse 1 + 2 und Klasse 4 + 5: $\frac{\text{Diff}}{m_{\text{Diff}}} = 2{,}85$. Unter m sind die rein statistischen mittleren Fehler der Prozentsätze verstanden.

genauere cytologische Untersuchung durchgeführt haben, fast stets gefunden haben, daß ein dem X-Chromosom fehlendes Stück auch völlig fehlt. Außerdem wären unwahrscheinliche Annahmen nötig, wollte man die Ursache der Geschlechtsumwandlungen in Translokationen von X-Chromosomenstücken an Autosomen sehen.

Es interessiert nun die Frage, wie häufig in unseren Versuchen Geschlechtsumwandlungen und wie häufig überhaupt Verkürzungen des X-Chromosoms aufgetreten sind. Unter den 9173 Pflanzen, die wir aus bestrahlten Sporogonen aufgezogen haben, befanden sich 4187 Weibchen, 166 X-Männchen und 4820 Männchen ohne X-Chromosomenstück[1]. Beziehen wir die Anzahl der X-Männchen auf die Summe von Weibchen und X-Männchen, also auf die Gesamtzahl der aus Sporen mit X-Chromosomen hervorgegangenen Pflanzen, so ergibt dies $3{,}81 \pm 0{,}29$ % X-Männchen. Berücksichtigen wir nur diejenigen Pflanzen, die aus den Sporogonen hervorgegangen sind, aus denen wir die 70 X-Männchen zum Vergleich ihres X-Chromosoms mit dem der Vergleichsweibchen gewählt haben, so bekommen wir unter 2572 Pflanzen 990 Weibchen, 77 X-Männchen und 1505 Männchen ohne X-Chromosomenstück. Für diese Sporogone bekommen wir also $7{,}2 \pm 0{,}8$% X-Männchen, bezogen auf die Anzahl der Pflanzen mit X-Chromosom.

Von den 70 cytologisch geprüften Vergleichsweibchen ließen, wie weiter oben schon gesagt wurde, 38, das sind $54{,}2 \pm 5{,}9$% eine Verkürzung des X-Chromosoms erkennen. Dazu sind die durch eine cytologisch nachweisbare Verkürzung des X-Chromosoms zustande gekommenen Umwandlungsmännchen zu zählen, also 88,6% (die Häufigkeit der X-Männchen mit cytologisch nachweisbarem X-Chromosomenstückverlust; s. oben) von der in diesem Material festgestellten Häufigkeit der X-Männchen, also von $7{,}2 \pm 0{,}8$%, das sind etwa 6,4%. Wir können also für dieses Material damit rechnen, daß in etwa 60% der Fälle eine sichtbare Chromosomenmutation des X-Chromosoms erfolgte. Wenn wir voraussetzen, daß auch bei den X-Männchen, bei denen sich cytologisch kein Verlust eines X-Chromosomenstückes nachweisen ließ, die Geschlechtsumwandlung durch einen kleinen Stückausfall im X-Chromosom bedingt wurde und ferner berücksichtigen, daß wir wahrscheinlich machen konnten, daß je kleiner der Stückausfall ist, desto unwahrscheinlicher dadurch

[1] Auf das Vorkommen einiger Pflanzen, die sowohl Archegonien als auch Antheridien tragen und die nach meinen Erfahrungen durchweg als Chimären zu deuten sind, sei an dieser Stelle nicht eingegangen. Sie sind in den genannten Zahlen mit inbegriffen. Vgl. meine früheren Angaben über solche Scheinsynöcisten (2). Lorbeer berichtet neuerdings über synöcische Gametophyten (5), doch wage ich zu bezweifeln, daß es sich in den von ihm besprochenen Fällen, die er nach Röntgenbestrahlung weiblicher Gametophyten beobachtet hat, wirklich um synöcische Formen handelt. Lebendes Material eines angeblichen Synöcisten, um das ich Herrn Dr. Lorbeer gebeten hatte, um seine Auffassung nachprüfen zu können, stellte er mir leider nicht zur Verfügung. Ein fixiertes Thallusstück, das er mir statt dessen übersandte, konnte meine Vermutung, daß es sich um eine ähnliche Chimäre handelt, wie sie in meinen Versuchen häufig aufgetreten sind, nicht entkräften.

eine Geschlechtsumwandlung bewirkt wird, so werden wir damit rechnen können, daß in dem genannten Material in mindestens 68% der Fälle $\left(\frac{54{,}2 \cdot 100}{88{,}6} + 7{,}2\,\%\right)$, wahrscheinlich aber noch häufiger, eine Chromosomenmutation des X-Chromosoms erfolgte. Für das Gesamtmaterial ergibt sich, wenn man als Maßstab für die Häufigkeitsbeziehungen die im Gesamtmaterial und die in den ausgewählten Sporogonen festgestellten Häufigkeiten der X-Männchen vergleicht, eine etwas mehr als halb so große Häufigkeit der Chromosomenmutationen. Wahrscheinlich ist der Unterschied, der zwischen verschiedenen Teilen des Materials besteht, auf den Unterschied im Zeitpunkt, in dem die Bestrahlung erfolgte, zurückzuführen. An anderer Stelle wird eine nähere, durch tetradenanalytische Untersuchungen ergänzte quantitative Behandlung der Häufigkeiten der X-Männchen und der beobachteten Zahlenverhältnisse der Geschlechter durchgeführt werden.

In unseren unbestrahlten Kontrollen fanden wir unter insgesamt 3231 Pflanzen 1694 Weibchen, 0 X-Männchen und 1537 Männchen ohne X-Chromosomenstück.

Es hat uns natürlich überrascht, in unseren Versuchen bei Bestrahlung mit etwa 3200 r in einem so hohen Prozentsatz Mutationen des X-Chromosoms zu finden. Man wird daraus schließen müssen, daß das heterochromatische X-Chromosom viel weniger stabil ist als die Autosomen, für die Chromosomenmutationen wohl kaum in einer so großen Häufigkeit angenommen werden können. Es ergeben sich aus dieser Feststellung neue Probleme hinsichtlich des Wesens des Heterochromatins.

Wenn wir nachgewiesen zu haben glauben, daß durch den Verlust eines bestimmten Stückes des X-Chromosoms ein Umschlag des Geschlechts von weiblich nach männlich erfolgt, so ist damit selbstverständlich nicht widerlegt, daß nicht auch durch andere Ursachen, z. B. durch die *Abänderung* eines bestimmten Genes, das mit der übrigen genetischen Gesamtkonstitution zusammen eben die Entwicklung in die weibliche Richtung lenkt, also durch eine Genmutation, der Geschlechtsumschlag bewirkt werden kann. Es wäre also möglich, daß bei einem Teil der 88% X-Männchen, die keine Verkürzung des X-Chromosoms erkennen ließen,, tatsächlich auch keine Chromosomenmutation vorlag, sondern eine Genmutation, die den Geschlechtsumschlag bedingte, oder auch, daß bei einigen der X-Männchen, bei denen ein Chromosomenstückverlust nachgewiesen wurde, der Geschlechtsumschlag nicht durch diesen Stückverlust, sondern durch eine Genmutation verursacht war. Es besteht aber gar kein Anhaltspunkt für eine solche Annahme, und nachdem wir Chromosomenstückverluste als Ursache für die Geschlechtsumwandlung nachgewiesen haben, erscheint es vorläufig viel wahrscheinlicher anzunehmen, daß auch *alle* in unseren Versuchen beobachteten Geschlechtsumwandlungen auf diese Ursache zurückzuführen sind und daß in den Fällen, wo sich cytologisch kein Verlust eines X-Chromosomenstückes nachweisen ließ, das verlorengegangene Stück eben zu kurz ist, um cytologisch in der somatischen Mitose nachweisbar zu sein.

Für unser Wissen über den Geschlechtsbestimmungsmechanismus ist aber die Frage auch gar nicht wichtig, ob außer durch die Wegnahme eines bestimmten Genes oder einer Gengruppe im X-Chromosom auch durch die Mutation dieses Genes die Umwandlung eines Weibchens in ein Männchen hervorgerufen werden kann. Wichtig ist der Nachweis, daß ich dadurch, daß ich vom X-Chromosom etwas entferne, eine genetische Konstitution erziele, die nun, genau wie die durch 7 Autosomen + dem Y-Chromosom gegebene genetische Konstitution, die Entwicklung in „männliche Bahnen" lenkt. Dies beweist zwar nicht, daß wir durch Entfernung dieses X-Chromosomenstückes genau dieselbe genetische Konstitution hinsichtlich der Geschlechtsbestimmung haben, wie sie im normalen männlichen Chromosomensatz 7 + Y gegeben ist, d. h. daß das X-Fragment dieselbe geschlechtsbestimmende Wirkung ausübt wie das Y-Chromosom, wohl aber daß die Wirkung von 7 + X-Fragment auf derselben Seite eines für die Entscheidung „weiblich oder männlich" kritischen physiologischen Punktes liegt wie die Wirkung von 7 + Y. Ich kann also die männliche Wirkung hervorrufen, indem ich von dem Genbestand, der die weibliche Wirkung hat, etwas wegnehme, ohne wieder etwas zufügen zu müssen. Ob es auch möglich ist, eine männliche Pflanze mit 7 + X-Fragment oder eine solche mit 7 + Y durch weitere Gen- oder Chromosomenmutationen genetisch so zu verändern, daß sie sich nun wieder weiblich entwickelt, wissen wir nicht, es erscheint aber nicht wahrscheinlich. Wir können deshalb auch nichts darüber aussagen, welche Gene oder ob überhaupt Gene in 7 + X-Fragment oder in 7 + Y diejenigen entwicklungsphysiologischen Bedingungen zu beeinflussen vermögen, deren Verschiebung die Entwicklung entweder in weibliche oder in männliche Bahnen lenkt.

Wie schon früher berichtet wurde (Knapp, 2, 3) sind die X-Männchen, die kein Y-Chromosom besitzen, steril; die Spermatozoiden sind nicht oder nur schwach beweglich. Lorbeer hat bei seinen Umwandlungsmännchen inzwischen dieselbe Feststellung gemacht. Als wahrscheinliche Ursache für diese Sterilität habe ich damals das Fehlen eines Y-Chromosoms genannt und dabei auf die Parallele mit *Drosophila* hingewiesen, wo bekanntlich im Y-Chromosom Fertilitätsfaktoren nachgewiesen sind. Lorbeer hat nun über Pflanzen berichtet, in denen ein „X♂-Chromosom" und außerdem ein Y-Chromosom zusammen in einer Zelle vorliegen und die bewegliche Spermatozoiden bilden (5). Auch wir haben inzwischen fertile X-Männchen mit einem überzähligen Y-Chromosom gefunden. Damit kann meine frühere Vermutung, daß die Sterilität der gewöhnlichen X-Männchen durch das Fehlen des Y-Chromosoms bedingt ist, als bewiesen gelten.

Unsere Befunde über die Geschlechtsumwandlung bei *Sphaerocarpus* stehen also in wesentlichen Punkten im Gegensatz zu den Ergebnissen, zu denen Lorbeer auf Grund seiner Untersuchungen kommt. Wir glauben festgestellt zu haben, daß Gametophyten, denen ein Stück des X-Chromosoms fehlt, lebensfähig sind und daß der Verlust eines

bestimmten Stückes des X-Chromosoms eine Umwandlung des Geschlechts bedingt, während LORBEER die Lebensunfähigkeit von Gametophyten, denen ein Stück des X-Chromosoms fehlt, behauptet und die Geschlechtsumwandlung auf die Umwandlung eines einzigen Realisatorgens γ in α zurückführt. Wir können uns diesen Gegensatz nicht anders als durch Beobachtungsfehler bei LORBEER erklären. Deshalb erscheint es auch zwecklos, auf weitere Behauptungen LORBEERs über Fragen der Geschlechtsbestimmung einzugehen, die zum Teil auf die genannten von uns angezweifelten Ergebnisse LORBEERs gegründet sind, zum Teil uns aus anderen Gründen äußerst unwahrscheinlich erscheinen. Wir behalten uns vor, nach Erscheinen des von LORBEER angekündigten ausführlichen Berichts auf einige Fragen zurückzukommen.

4. Zusammenfassung.

1. Gametophyten von *Sphaerocarpus Donnellii*, die ein Stück des heterochromatischen X-Chromosoms völlig verloren haben, sind lebensfähig.

2. Der Verlust eines bestimmten Stückes des X-Chromosoms im normalen Chromosomensatz bedingt, daß statt weiblicher Gametophyten männliche gebildet werden. Es konnte nicht näher festgelegt werden, welches Stück des X-Chromosoms verlorengehen muß, damit eine Geschlechtsumwandlung erfolgt.

3. Solche „X-Männchen" sind nicht fertil. Durch Zufügung eines Y-Chromosoms können sie fertil werden. Das Vorhandensein eines Y-Chromosoms scheint also für die Fertilität notwendig zu sein.

4. Die Erwartung dafür, daß eine Geschlechtsumwandlung durch den Verlust eines X-Chromosomenstückes bedingt wird, ist um so höher, je größer das verlorene Chromosomenstück ist. Dies wird dadurch erklärt, daß rein zufallsgemäß in einem größeren Chromosomenstück auch das für das Geschlecht entscheidende Chromosomenstück mit größerer Wahrscheinlichkeit enthalten sein muß als in einem kleinen.

5. Die Häufigkeit der X-Männchen nach Bestrahlung mit 3200 r, bezogen auf die Gesamtzahl der Pflanzen mit X-Chromosom, betrug für unser ganzes daraufhin untersuchtes Material 3,8%, für einen bestimmten Teil unseres Materials 7,2%. Für die Häufigkeit von Chromosomenmutationen des X-Chromosoms kann man in dem daraufhin geprüften Teil unseres Materials mit mindestens 68% der X-Chromosomen rechnen.

Literatur.

1. **Heitz, E.:** Chromomosenstruktur und Gene. Z. Abstammgslehre **70**, 402 bis 447 (1935). — 2. **Knapp, E.:** Untersuchungen über die Wirkung von Röntgenstrahlen an dem Lebermoos *Sphaerocarpus*, mit Hilfe der Tetraden-Analyse. I. Z. Abstammgslehre **70**, 309—349 (1935). — 3. **Knapp, E.:** Zur Frage der genetischen Aktivität des Heterochromatins, nach Untersuchungen am X-Chromosom von *Sphaerocarpus Donnellii*. Ber. dtsch. bot. Ges. **53**, 751—760 (1935). — 4. **Lorbeer, G.:** Die Umwandlung eines haploiden, genotypisch weiblichen Gametophyten von *Sphaerocarpus Donnellii* in einen männlichen mit Hilfe von Röntgenstrahlen. Planta (Berl.) **25**, 70—83 (1936). — 5. **Lorbeer, G.:** Über das Vorkommen von drei verschiedenen Geschlechtsrealisatoren bei den Lebermoosen. Planta (Berl.) **27**, 708—717 (1938).

Erklärung zu Tafel 1.

Neben der Tafel Strichzeichnungen der einzelnen Präparate, die die Orientierung erleichtern und die gegebene Deutung zeigen sollen. Der Pfeil zeigt überall, soweit im folgenden nichts anderes vermerkt ist, auf die Spindelfaseransatzstelle des X-Chromosoms. Die (nicht immer im ganzen Verlauf erkennbaren) schwachgefärbten euchromatischen Autosomen in der Prophase sind durch punktierte Umrisse angedeutet. Die jeweils in Klammern gesetzten Bezeichnungen sind die Bezeichnungen der betreffenden Pflanze, des Präparates und der Photographie. Vergr. überall 1 : 2000.

1. Metaphase aus normalen Männchen, 7 + Y. Das Y-Chromosom fast punktförmig (211 ② D 188 a, ♂. — . EK 325).

2. Interkinese. X-Chromosom ohne nachweisbare Verkürzung. Autosomen kaum erkennbar (1096 ⑮ D 29 a, ♀. 4571 a, 1. MA 778 a).

3. Prophase. X-Chromosom ohne nachweisbare Verkürzung. Autosomen angedeutet (1096 ⑮ C 36 b, ♀. 4511 a, 2. MA 745).

4. Prophase. X-Chromosom ohne nachweisbare Verkürzung. Autosomen (gespalten) großenteils erkennbar. Oben stärker gefärbtes Endchromomer eines Autosoms (1096 ⑨ D 91 a, ♂. 4643 a, 6. MA 780 b).

5. Prophase. X-Chromosom ohne nachweisbare Verkürzung. Auch alle 7 Autosomen deutlich zu erkennen, aber nicht alle in der Ebene der Photographie. Die dunklere Stelle rechts oben nicht Heterochromatin, sondern Schleife in einem euchromatischen Autosom (1096 ⑮ D 4 a, ♀. 4544 a, 2. MA 762 c).

6. Metaphase. X-Chromosom ohne nachweisbare Verkürzung (1096 ⑯ C 65 a, ♀. 4618 a, 1. MA 782 b).

7. Metaphase. X-Chromosom ohne nachweisbare Verkürzung (1096 ⑨ D 26 a, ♀. 4567 a, 2. MA 776 a).

8. Anaphase. X-Chromosom ohne nachweisbare Verkürzung. In Skizze nur X-Chromosomen eingezeichnet. (1096 ⑧ D 134 b, ♂. 4528 a, 1. MA 749).

9. Prophase. X-Chromosom mit etwas verkürztem Schenkel. Autosomen (gespalten) erkennbar (1096 ⑮ D 17 a, ♀. 4545 a, 2. MA 758).

10. Prophase. X-Chromosom mit deutlich verkürztem Schenkel (1096 ⑧ D 137 a, ♂. 4527 1, 1. MA 748).

11. Prophase. X-Chromosom mit deutlich verkürztem Schenkel (1096 ⑮ D 39 c, ♀. 4549 b . MA 764).

12. Prophase. X-Chromosom mit stark verkürztem Schenkel (1096 ⑮ D 38 a, ♀. 4548 a, 1. MA 763).

13. Interkinese. X-Chromosom mit deutlich verkürztem Schenkel (1096 ⑨ C 14 a, ♀. 4516 a, 1. MA 760).

14. Prophase. Dieselbe Pflanze (4516 a, 4. MA 761 b).

15. Dasselbe Präparat, andere Einstellung (MA 761 a).

16. Anaphase (nur die eine Hälfte). In der Skizze nur das X-Chromosom gezeichnet. Dieselbe Pflanze (4516 a, 2. MA 759 b).

17. Prophase. X-Chromosom stark verkürzt, mit sekundärer Einschnürung. X-Chromosom und Autosomen gespalten (1096 ⑮ D 62 b, ♀. 4551 a, 4. MA 768d).

18. Metaphase. Dieselbe Pflanze. Auch hier sekundäre Einschnürung zu erkennen (4551 a, 7. MA 772).

19. Anaphase. Dieselbe Pflanze. In der Skizze nur X-Chromosomen gezeichnet (4551 a, 3. MA 767 a).

20. Anaphase, gequetscht und in der Lage gestört. Dieselbe Pflanze. Sekundäre Einschnürung deutlich. In der Skizze nur X-Chromosomen gezeichnet (4551 a, 1. MA 765 c).

21. Prophase. X-Chromosom mit stark verkürztem Schenkel (1096 ⑮ D 114 a, ♂. 4583 a, 1. MA 1116 d).

22. Dasselbe Präparat, andere Einstellung (MA 1116 e).

23. Metaphase. Dieselbe Pflanze (4583 a, 3. MA 777).

24. Prophase. Heterochromatisches X-Chromosomenstück an euchromatisches Autosom transloziert *(T)*. Im übrigen Unklarheiten im Präparat (1096 ⑮ D 67 b, ♂. 4693 a. MA 1126 b).

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ÜBER DIE ROLLE DER DESOXYRIBOSENUKLEINSÄURE BEI DER ZELLTEILUNG

Von

T. CASPERSSON.

Mit 7 Textabbildungen (13 Einzelbildern.)

(Eingegangen am 2. Januar 1939.)

Während der Zellteilung sind große Nukleinsäuremengen in den Chromosomen angesammelt. Die Rolle der Nukleinsäure im Chromosom ist noch unklar. Einen Weg zum Studium dieses Problems scheint die genaue Untersuchung der Zeitverhältnisse während der prophasischen Nukleinsäureanreicherung und der Chromosomenentwicklung zu weisen.

Durch Messung des Absorptionsspektrums von Zelleinzelheiten kann ihr Gehalt an ultraviolett-absorbierenden Stoffen bestimmt werden. Der theoretische Hintergrund dieser Methode, sowie ihre Verwendbarkeit ist schon früher dargestellt worden (CASPERSSON 1936 I). Im Prinzip arbeitet die Methode so, daß die Lichtabsorptionen des mikroskopischen Objekts in einer Serie verschiedener Wellenlängen bestimmt werden. Die Form der dabei entstehenden Absorptionskurve gibt über die in das Objekt eingehenden Stoffe Aufschluß, ihre Höhe ergibt die Menge dieser Substanzen. Eine besonders hohe spezifische Absorption in Ultraviolett haben die Nukleinsäuren, welche durch ihren Gehalt an absorbierenden Pyrimidinringen bedingt ist. Diese Absorption ist so hoch, daß mittels Messung in der Zelle Konzentrationen bis zu 1% in 1μ dicken Schichten gemessen werden können.

Untersuchungen verschiedener Stadien der Zellteilung mit dieser Methode haben ergeben, daß während der Metaphase die Nukleinsäure elektiv in den Chromosomen lokalisiert ist, die dann an dieser Säure besonders reich sind. Orientierende Untersuchungen mittels Ultraviolettabsorptionsmessung, an einer Serie verschiedener Untersuchungsobjekte, wiesen darauf hin, daß der Zellteilung eine Ansammlung oder Synthese von Nukleinsäuren in den sich ausbildenden Chromosomen vorangeht (CASPERSSON l. c.). Dadurch werden auch in der Interphase sehr nukleinsäurearme Kerne in der Prophase reich an diesen Substanzen. In der darauffolgenden Telophase verarmen die Kerne an Nukleinsäuren. Von mehreren Autoren ist vor der Teilung eine Zunahme der mit basischen Farbstoffen färbbaren Substanzen beobachtet worden, was als ein gleichartiges Phänomen betrachtet werden muß. Literaturangaben in dieser Hinsicht enthält die bereits zitierte Arbeit.

Die zentralen Prozesse vor der Zellteilung sind die Teilung der Erbsubstanz und die Kondensation und Kontraktion der Chromosomen.

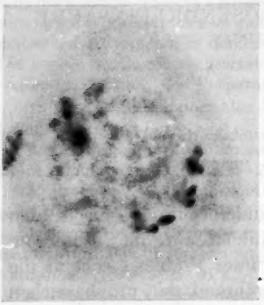
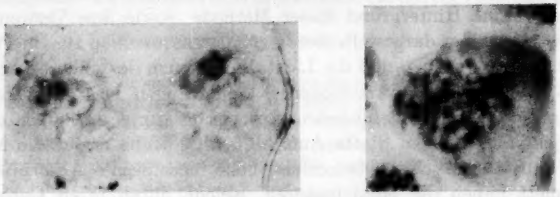


Abb. 1. *Myrmus miriformis.* Spermatocyte, lebende Zelle. Wellenlänge 2750 Å. Vergr. 2500fach.

Der Nachweis des Charakters der Thymonukleinsäure als hochpolymere Verbindung mit sehr langgestreckten Molekülketten (Signer, Caspersson und Hammarsten 1938) sowie der Nachweis einer Übereinstimmung in der Periode im Molekel mit der einer gestreckten Polypeptidkette (Astbury und Bell 1938) zeigt, daß sie außerordentliche Eignung dafür besitzt, ein wichtiger Faktor für den molekularen Mechanismus zu sein. Daß zwischen Genvermehrung und Nukleinsäureumsatz gewisse Beziehungen existieren, ist gezeigt worden (Caspersson und Schultz 1938).



a b

Abb. 2a und b. *Myrmus miriformis.* Junge (a) und ältere (b) Spermatocyten I, vgl. Text. Wellenlänge 2750 Å. Vergr. 1480fach.

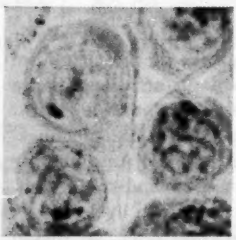


Abb. 3. *Myrmus miriformis.* Verschiedene Stadien von Spermatocyten I nebeneinanderliegend. Lebendaufnahme. Wellenlänge 2750 Å. Vergr. 920fach.

Die meiotische Prophase bietet eine geeignete Gelegenheit zum Studium der evtl. zeitlichen Korrelation zwischen den beiden obengenannten Prozessen und der Nukleinsäuresynthese im Chromosom (vgl. unten). In der Regel ist diese Prophase lang genug, um die Entwicklung der Chromatiden und ihre spätere Kontraktion zu kompakten Metaphasenchromosomen gut verfolgen zu können. Bei Heuschrecken sind diese Verhältnisse zuletzt vor allem von Belar (1929) dargelegt. Hier ist auch die Verteilung der Thymonukleinsäure in den Zellstrukturen während der verschiedenen Teilungsphasen (Caspersson 1936) beschrieben worden. Die Lokalisation der Säure ist mittels Ultraviolettabsorptionsmessung und durch

Digestionsversuche bei einer *Chorthippus*- und einer *Gomphocerus*-Art untersucht worden.

Der weiteren Kontrolle wegen sind jetzt, außer dem größeren Material dieser beiden Arten, auch eine andere *Chorthippus*- und zwei andere *Gomphocerus*-Arten nach denselben Methoden untersucht worden. Alle Ergebnisse stimmten, von kleineren Artverschiedenheiten abgesehen, mit dem früher angegebenen Schema überein. Sie bleiben deshalb

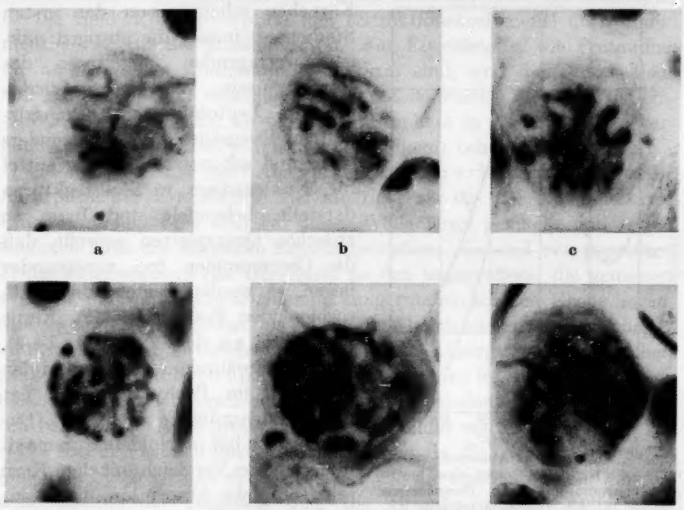


Abb. 1a—f. *Gomphocerus (maculatus?)*. Spermatocyten in verschiedenen Stadien, vgl. Text. Wellenlänge 2750 Å. Vergr. 1220fach.

hier unbesprochen. Auch die Hemiptere *Myrmus miriformis*, deren Spermatogenese von T. Ekblom (1934) beschrieben worden ist, wurde an Hand größeren Materials untersucht. Sie folgte hinsichtlich der Nukleinsäureverteilung ganz dem gleichen Schema.

Der Verlauf der Veränderungen während der Prophase zur ersten Spermatocytenteilung zeigt sich, in Kürze dargestellt, wie folgt: Im frühesten Leptotänstadium, welches rasch durchlaufen wird, wächst der Kern schnell und ist im Absorptionsmaximum der Nukleinsäuren schwach absorbierend. Die absorbierende Substanz ist in sehr feinen Körnchen angesammelt (vgl. Abb. 1). In späteren Stadien ist die Allgemeinabsorption des Kernes höher (Abb. 2—4); die Körnchen scheinen größer zu sein und die Anordnung in Reihen kommt zum Vorschein. Im weiteren Verlaufe wird diese Erscheinung immer deutlicher, und es beginnen die

Körnchenreihen sich zusammenzuziehen. Diese Kontraktion setzt sich bis zur Ausbildung der kompakten Metaphasenchromosomen fort.

Die ultraviolettabsorbierenden Körnchen sind die Träger der Nukleinsäurekomponente. Abb. 5 zeigt das Absorptionsspektrum des zentralen Gebietes eines ausgebildeten Metaphasenchromosoms der einen *Gomphocerus*-Art. Die Absorptionskurve zeigt ein gut ausgebildetes Nukleinsäuremaximum, welches über einer niedrigen Eiweißkurve liegt. Die Körnchen scheinen von den ersten Stadien ab immer die einzigen nukleinsäuretragenden Strukturen des Kernes zu sein. Wird eine Spermatocyte im Leptotän mit lanthansalzhaltiger Essigsäure, die ein besonders gutes und schonendes Fällungsmittel für Nukleinsäure in Zellstrukturen darstellt, behandelt und dann so zwischen Quarzplatten gepreßt, daß die Chromosomen frei voneinander liegen, so werden keine anderen absorbierenden Bestandteile im Kerne beobachtet als diese (vgl. Abb. 1—4). Die Nukleinsäurekonzentration in den Körnchen im Pachytänstadium hat die Größenordnung 5—10%. Das Cytoplasma hat im Nukleinsäuremaximum eine im Vergleich mit dem Kern sehr schwache Absorption.

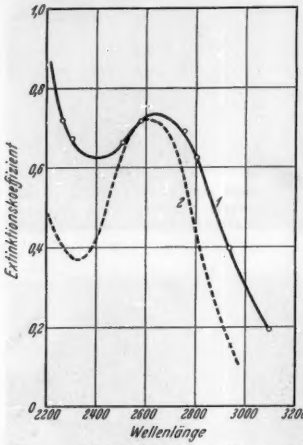


Abb. 5. Ultraviolett-Absorptionsspektrum vom zentralen Gebiet eines Metaphasenchromosoms von *Gomphocerus* (Kurve 1). Als Vergleich ist eine Absorptionskurve von Thymonukleinsäure (Kurve 2) mit eingezeichnet.

Daß gerade die Chromomeren die Träger der Nukleinsäurekomponente sind, scheint ein allgemeines Phänomen zu sein. Die Nukleinsäure im Kern während der Prophase wird anscheinend immer in den sich ausbildenden Chromosomen angesammelt. Sie geht in toto in die Metaphasenchromosomen ein. Besonders auffallend sind diese Phänomene in nukleinsäurearmen Kernen wie in gewissen Epithelien. In allen diesen Stadien geben die Chromosomen eine starke FEULGEN-Reaktion, was auf die Anwesenheit von Desoxyribosenukleinsäure hindeutet. Da diese Säuren bis jetzt nicht mit Sicherheit außerhalb des Zellkerns nachgewiesen worden sind, scheinen sie die typischen „Chromosomnukleinsäuren" zu sein. Wir sind mit großer Wahrscheinlichkeit berechtigt, von einer Synthese der Desoxyribosenukleinsäure an der Chromatide zu sprechen. Von gewissem Interesse, wenn auch sehr geringem Beweiswert, ist in diesem Zusammenhange die Beobachtung, daß bei Heuschrecken in der frühen meiotischen Prophase die Chromomeren

lebender Zellen gegen die Umgebung oft unscharf abgegrenzt, in späteren Stadien aber mit einer distinkten Oberfläche erscheinen.

Das Absorptionsmaximum der Nukleinsäuregruppe liegt bei 2600 Å (vgl. Abb. 5). Die Absorptionsmaxima der zyklischen Aminosäuren liegen bei etwas höheren Wellenlängen, zwischen 2750 und 2900 Å. Sie haben in der Regel ein Minimum um 2600 Å. Die spezifische Absorption der Eiweißstoffe ist je nach ihrem Gehalt an diesen Säuren verschieden. Sogar bei dem sehr stark absorbierenden Serumalbumin ist bei 2600 Å die Absorption (der Extinktionskoeffizient) noch 40mal niedriger als die der Nukleinsäuren. Für Eiweißstoffe, wie Protamine, die an zyklischen Aminosäuren sehr arm sind, sind die Unterschiede viel größer, bis zum 400fachen. In der Zelle werden also die Nukleinsäuren, besonders wenn sie wie in der Prophase in großen Mengen vorhanden sind, das Absorptionsbild vollkommen beherrschen.

Auch in der Nähe ihrer Absorptionsmaxima, z. B. bei 2750 Å, ist die Absorption der Eiweißstoffe viel niedriger als die der Nukleinsäuren. Die Aufnahmen 1—4 sind bei dieser Wellenlänge gemacht und zeigen demnach auch die schwache Eiweißabsorption. Sie sind von gepreßten Zellen aufgenommen (im Gegensatz zu den ungepreßten, die gemessen wurden), und da die Chromonemata einigermaßen in einer Ebene liegen, sind sie soweit vergleichbar, als sie den oben bezeichneten großen Gehaltsunterschied an Substanzen mit Nukleinsäureabsorption, der zwischen ganz jungen und älteren Spermatocyten vorhanden ist, aufzeigen. Dieser Unterschied ist bei verschiedenen Arten verschieden. Bei *Gomphocerus* und bei *Myrmus* ist er groß (vgl. auch die Bilder von Ekblom l. c.). Da dieses Stadium schnell durchlaufen wird und da die kleinen Spermatogonien nicht so einfach zu messen sind wie die Spermatocyten, können keine bestimmten Angaben über die Ursache dieser Unterschiede zwischen den verschiedenen Rassen gemacht werden.

Die oben angeführten Faktoren zusammengenommen ergeben, daß die totale Absorption bei 2600 Å in dem Spermatocyten-Prophasekern ein gutes Maß des Nukleinsäuregehalts abgibt. (Bei diesen Zellen sind die Verhältnisse besonders günstig. Die direkten Ultraviolettaufnahmen von Kernen im allgemeinen müssen sehr vorsichtig beurteilt werden. Die Ultraviolettabsorption, auch bei 2600 Å, sagt nichts über den Nukleinsäurecharakter einer Zelleinheit aus. Erst die Messung in verschiedenen Wellenlängen läßt Genaueres darüber erkennen.)

Es ist natürlich am vorteilhaftesten, bei solchen Messungen mit lebenden Zellen zu arbeiten. Deshalb sind solche auch immer benutzt worden. Praktisch wurde die Messung so ausgeführt: Der Heuschreckenhoden wurde auspräpariert und einige von den Hodenschläuchen auspräpariert. Sodann wurden sie mit einem Tropfen Ringerscher Lösung auf die Quarzplatte gebracht. Die Schläuche wurden mit Nadeln geöffnet. Hiernach wurde das Quarzdeckglas aufgelegt, wobei kontrolliert

wurde, ob die Zellen nicht etwa gepreßt wurden. Das Präparat wurde nunmehr in ein Mikroskop mit optischer Ausrüstung für Ultraviolett nach KÖHLER eingeführt und mit einem Bündel von monochromatischem ultraviolettem Licht mit niedriger Apertur beleuchtet. Das Bild wurde dann über ein lichtmessendes System projiziert. Über die Prinzipien der Behandlung der optischen Fragen bei der Messung wird auf die früher zitierte Arbeit hingewiesen. Für die Messung der vom Objekt durchgelassenen Lichtmengen wurde eine photoelektrische Anordnung benutzt. Das Bild des Gegenstandes wurde über eine ultraviolettempfindliche

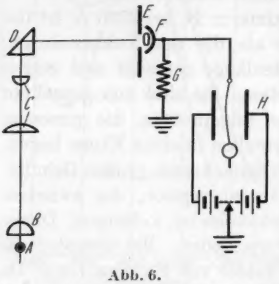


Abb. 6.

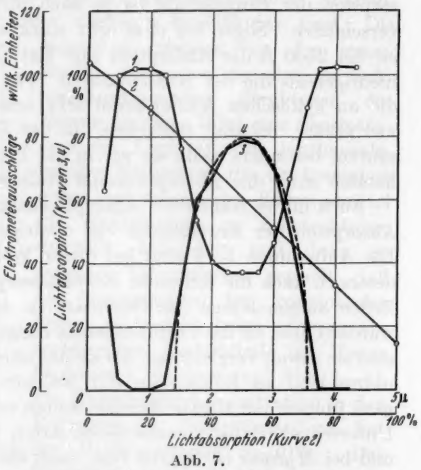


Abb. 7.

Abb. 6. Prinzipschema des Meßverfahrens. Das Objekt *A* wird mit einem engen Bündel von monochromatischem ultraviolettem Licht beleuchtet. Ein Bild wird vom Ultraviolettmikroskop (*B* ist das Objekt und *C* das Okular) auf die Lochblende *E* aufgeworfen. *D* ist ein bewegliches Prisma, mit welchem das Bild über die hinter dem Loch angebrachten Photozelle *F* bewegt werden kann. *G* ist ein Hochohmleckwiderstand. Mit dem Saitenelektrometer *H* wird die, vom Lichtstrom auf *F* abhängige Spannung des Photozellsystems gemessen.

Abb. 7. Eine typische Meßkurve. Kurve 1 gibt in willkürlichen Einheiten die Elektrometerausschläge die bei der Messung quer über die Zelle längs einem Durchmesser erhalten werden, an. Die Eichkurve des Elektrometers, 2, gibt den Zusammenhang zwischen den Ausschlägen und der Lichtabsorption. Damit werden die Werte von 1 in Kurve 3 überführt, die die Absorption in Prozent in den verschiedenen Meßpunkten angibt. Die punktierte Kurve 4 ist die graphisch interpolierte Kurve für eine absorbierende Kugel.

Photozelle projiziert, der Photostrom mit einem Saitenelektrometer gemessen. Die Öffnung der Photozelle soll dabei im Vergleich zum Durchmesser des Kernes klein, jedoch im Verhältnis zur Teilchengröße groß sein. In der Regel wurde eine Öffnung von $^1/_{20}$ des Kerndurchmessers verwendet. Mittels einer Prismenanordnung wurde das Bild, einem Zelldiameter entlang, über die Photozellöffnung fortbewegt. Aus den Werten des Photostromes, die verschiedenen Bildpunkten entsprachen, wurde die Durchlässigkeit in etwa 30 Punkten auf diesem Durchmesser berechnet. Abb. 6 zeigt das Prinzip und Abb. 7 eine Meßkurve dieser

Art. Die gefundenen Werte wurden dann in Extinktionskoeffizienten übergeführt.

In dem meiotischen Prophasekern ist die Substanz, deren Menge gemessen werden soll, in winzig kleinen Körnchen vorzufinden (vgl. Abb. 1). Bis zum Anfang des Pachytänstadiums ist die Verteilung innerhalb des Kernes im großen und ganzen gleichförmig. Für einen Lichtstrahl, der ein in Hinblick auf die Absorption inhomogenes Medium durchläuft, ist die Extinktion gleich der Summe der Extinktionen der einzelnen Teilchen. Sie ist also von der Art der Verteilung der absorbierenden Substanzen längs des Weges des Lichtstrahles unabhängig. In den hier in Frage kommenden Stadien ist die Form des Zellkerns sphärisch. Unter der Voraussetzung, daß die Teilchen im Vergleich zum Kernradius klein und im ganzen Kern gleichmäßig verteilt sind, würde aus einem einzigen Meßwert längs einem Durchmesser die Gesamtabsorption des Kernes zu berechnen sein. Die wichtigste Voraussetzung dafür, daß durch die Messung ein genauer Wert für den wirklichen Nukleinsäuregehalt gefunden werden kann, ist, daß die absorbierenden Körnchen gleichmäßig verteilt sind. Dieser Faktor muß bei jeder Messung genau kontrolliert werden. Dies wird erreicht, indem man die Lichtabsorption der verschiedenen Meßpunkte und ihren Abstand vom Kernzentrum in ein Koordinatensystem einträgt (Abb. 7). Ist die Verteilung homogen, so muß die Form der erhaltenen Kurve mit der entsprechenden Kurve einer gleich großen homogen absorbierenden Kugel übereinstimmen. Deswegen wurde in sämtlichen Fällen die Absorptionskurve einer gleich großen homogenen Kugel, von gleicher Absorption im Zentrum, über die durch die Messung gewonnene Kurve eingezeichnet (Abb. 7). Die Abweichungen von dieser idealen Kurve diente so als empfindlicher Indikator für die Homogenität des gemessenen Kerns. Sind z. B. größere Mengen auf einer Seite des Kernes angehäuft, so wird die Meßkurve schief oder verschoben. Das ist in frühen Stadien bei *Myrmus* der Fall, wo die heteromatischen Stücke der Chromosomen nahe aneinander liegen und der Kern im Bereich der euchromatischen Chromosomenabschnitte noch nukleinsäurearm ist. Das heterochromatische X-Chromosom bei den Heuschrecken bedeutet mengenmäßig verhältnismäßig wenig. Im späteren Pachytän und Diplotän zeigen sich unregelmäßige Abweichungen der Kurven voneinander; die fortgeschrittene Chromatidenkontraktion macht die Messung dann unmöglich. Zeigen die beiden Kurven keine Abweichungen, die außerhalb des Bereiches der Meßfehler liegen, wird der Zentralwert der eingetragenen Kurve genommen und aus diesem sowie dem Kerndurchmesser wird dann die Totalabsorption bei der in Frage kommenden Wellenlänge ermittelt. Dieses Vorgehen ergibt zugleich auch eine Kontrolle der nächst wichtigen Fehlerquelle, nämlich der Abweichung von der sphärischen Kernform, soweit sie durch Druck des Deckglases entstehen kann. Eine solche

kommt zwar nicht in der photographischen Aufnahme zum Vorschein, zeigt sich aber als eine Abweichung von der idealen Sphärenkurve, da das Maximum der Kurve abgeflacht erscheint.

Die Messungen der Totalabsorption bei 2570 Å, die in diesem Falle einen guten Indikator für den Gesamtnukleinsäuregehalt ergaben, sind nur an der einen *Gomphocerus*-Art (mit höchster Wahrscheinlichkeit *G. maculatus*) durchgeführt worden, bei welcher die Nukleinsäureverteilung in der Meiose schon detailliert beschrieben ist (Caspersson 1936). Der Quotient zwischen den Extinktionskoeffizienten des Kernzentrums für 2570 und 2750 Å ergibt nach Bestimmung an einigen Zellen im frühen Leptotän und in den späteren Stadien dieselben Werte, wie die oben für das Metaphasenchromosom angeführten.

Abb. 7 zeigt die typische Meßkurve eines Kernes bei 2570 Å. Die Übereinstimmung zwischen den Formen der erhaltenen und der idealen Kurve ist bis zum Diplotän immer gut, von ganz vereinzelten Zellen abgesehen. Die Übereinstimmung ist auch im Pachytän erstaunlich gut, sogar in Stadien, in welchen die Aufnahmen — besonders die gewöhnlichen, von gepreßten Zellen — eine sehr inhomogene Verteilung vortäuschen. Von Zellen im frühen Pachytän sind Messungen in verschiedenen Azimuten mit guter Übereinstimmung der Werte durchgeführt worden. In den Stadien, in denen die Chromatidenkontraktion sehr weit vorgeschritten ist, besteht mit hoher Wahrscheinlichkeit eine solche Anordnung der Chromosomen im Kerne, daß die zentralen Absorptionswerte zu hoch werden. Es ist wichtig, darauf hinzuweisen, daß der dadurch entstandene Fehler eine scheinbare Erhöhung der Gesamtabsorption bedingen würde und daß die später nachgewiesene Konstanz des Nukleinsäuregehaltes nicht durch eine Fehlerquelle solcher Art vorgetäuscht werden kann.

Die Messungen sind direkt an unbehandelten Zellen ausgeführt worden. Die Lichtintensitäten sind so groß, daß während der Messung die Chromosomen unbedeutend quellen, was aber die Meßwerte fast gar nicht beeinflußt, weil ja die Extinktion in jedem Meßpunkte die Summe der Partialextinktionen ist. Wenn die lebende Zelle längere Zeit der Bestrahlung unterliegt, werden die Kernstrukturen diffus, quellen weiter und fließen zuletzt zusammen. Ein merkbarer Abbau der absorbierenden Nukleinsäuregruppen tritt jedoch erst viel später ein. Im aktuellen Falle wurde durch Wiederholung der Messungen mit identischen Ergebnissen gezeigt, daß diese Verhältnisse keine Rolle spielen.

In der Tabelle 1 ist d der Durchschnitt des Kernes in μ, k ist der dekadische Extinktionskoeffizient bei 2570 Å im zentralen Gebiet der graphisch interpolierten idealen Kugelkurve (vgl. oben). NS ist die gesamte Menge absorbierender Substanz in der Zelle, als Nukleinsäure in 10^{-9} mg berechnet.

Ist ε der Extinktionskoeffizient für 100 μ 1%iger Thymonukleinsäurelösung, so ist

$$NS = \frac{4\pi \cdot d^3}{3 \cdot 8} \cdot \frac{k}{\varepsilon \cdot d} \cdot 10^{-9} \text{ mg Nukleinsäure.}$$

Da ε gleich 2,1 ist, so wird

$$NS = 0{,}25 \cdot k \cdot d^2 \cdot 10^{-9} \text{ mg Nukleinsäure.}$$

Einige Daten von Diplotän sind in Klammern angeführt. Die Kontraktion ist soweit vorgeschritten, daß hier die Messungen nach der angewandten Technik anfangen, unzuverlässig zu werden.

Tabelle 1.

Stadium	d	k	NS 10^{-9} mg
Frühes Leptotän	13,1	0,456	19,6
	14,1	0,337	16,8
	13,5	0,390	17,8
	12,5	0,284	11,2
	12,9	0,398	16,6
Spätere Leptotänstadien (mit Amphitän) bis Anfang Pachytän	15,0	0,44	24,7
	10,6	0,796	22,4
	10,6	0,760	21,4
	13,0	0,585	25,5
	11,3	0,688	22,0
	18,1	0,300	24,3
	14,5	0,523	26,0
	10,0	0,796	20
(Synizese)	12,8	0,568	23,3
Pachytän bis Anfang Diplotän	12,0	0,699	25,2
	13,5	0,482	21,8
	11,9	0,658	23,4
	11,3	0,796	25,4
	13,5	(0,585	26,5)
	11,5	0,620	20,5
	14,1	0,522	26,0
Diplotän	(15	0,509	28,4)
	(14	0,45	22,2)
	(17,5	0,347	26,5)

Von den immer blassen ersten Stadien (vgl. die Abbildungen) der Spermatocyten, die schnell durchlaufen werden, sind keine Messungen gemacht worden. In den jüngsten gemessenen Zellen ist schon die Reihenanordnung der Chromomeren deutlich und die Absorption (siehe die k-Werte) des Kerns ist schon so groß, daß er auf der photographischen Platte viel dunkler als die in den Figuren reproduzierten ersten Stadien erscheint.

Für die späteren Stadien zeigt die Tabelle, daß die Nukleinsäuremenge im Zellkern während der ganzen Zeitperiode vom mittleren Leptotän bis zum Diplotän, welche der Periode der starken Chromatidenkontraktion entspricht, stets dieselbe ist.

Die Nukleinsäuresynthese in den Chromatiden ist also vor Beginn der eigentlichen Chromosomenkontraktion abgelaufen und scheint diese also nicht zu berühren. Wenn die Kontraktion sich später vollzieht, so muß sie von anderen Faktoren ausgelöst werden. Ob die schon vorhandenen Nukleinsäuren dabei sekundär eine Rolle spielen, sei dahingestellt. Würden die Nukleinsäuren für den Kontraktionsmechanismus eine Unterlage darstellen, wäre zu erwarten, daß auch in früheren Stadien der Meiose mit langen Chromonemata, in welchen die Chromomeren weit voneinander liegen, zwischen ihnen nukleinsäurehaltige Verbindungsstrecken vorhanden sein sollten. Im hier untersuchten Material sind solche aber nicht beobachtet worden. Dies steht in voller Analogie mit den Riesenchromosomen der Dipteren, bei welchen mit Absorptions-

messungen und Digestionsmethoden keine Nukleinsäurekomponente in den Chromosomenteilen zwischen den Querscheiben nachzuweisen war (untersucht sind *Chironomus* und *Drosophila*, CASPERSSON 1936).

Weiter besteht die Frage, ob die Thymonukleinsäureansammlung mit der Genreduplikation in Zusammenhang stehen könnte. Zeitlich scheinen die beiden Prozesse übereinstimmen zu können. Der Chromosomenspalt wird schon in der frühen Prophase sichtbar. Bei allen gebräuchlichen Chromatinfärbungen, die mit basischen Farbstoffen in hohen Konzentrationen und in der Regel auch in saurer Lösung gemacht werden sowie auch bei der gewöhnlichen Ausführung der Chromatinfärbung mit Hämatoxylin, verleiht jedoch gerade die Thymonukleinsäure dem Chromonema die Färbbarkeit. Die Beobachtung einer sehr frühen prophasischen Spalte besagt demnach nichts gegen einen solchen Mechanismus.

Die anscheinend außerordentlich spezifische Lokalisation der Desoxyribosenukleinsäure auf den genomatischen Elementen sowie die universelle Verbreitung dieser Substanzen in Tier- und Pflanzenwelt, können in derselben Richtung gedeutet werden. Sie sind ja in allen untersuchten höheren Pflanzen und Tieren nachgewiesen worden, ebenso auch im Zusammenhange mit den Teilungen bei niederen Organismen, wie Hefezellen und Bakterien.

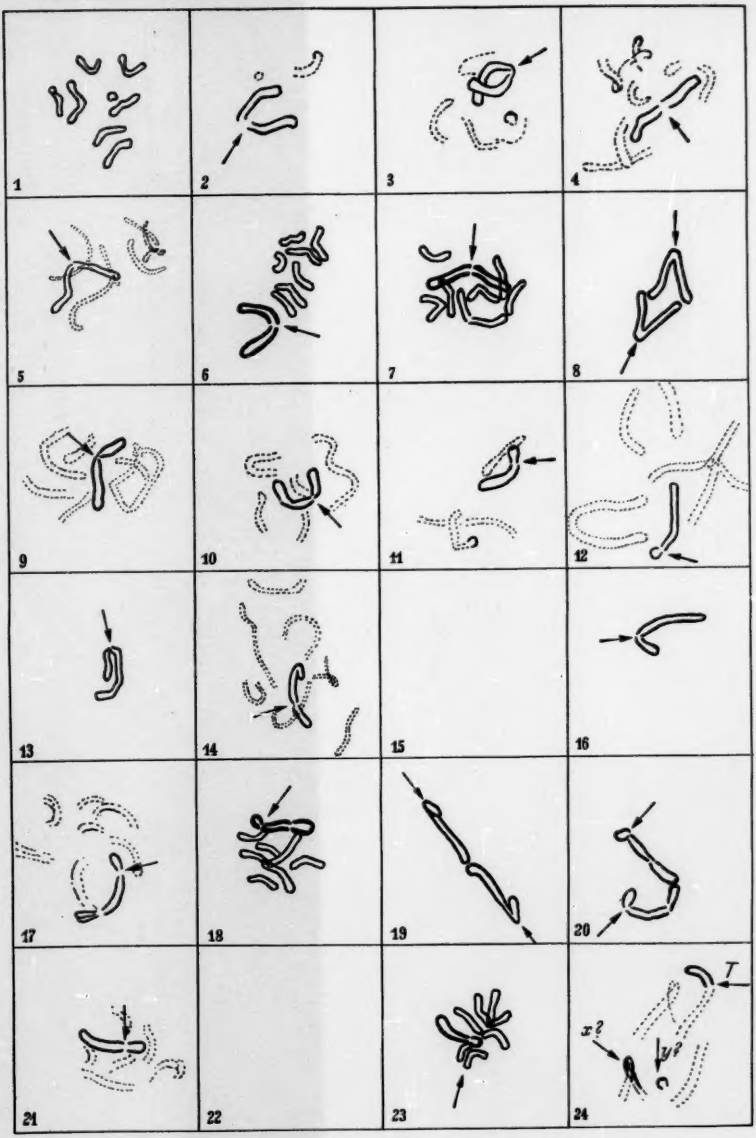
Zusammenfassung.

Die Desoxyribosenukleinsäuren scheinen für die Zellteilung notwendig zu sein. Es ist schon früher an Hand einigen Materials gezeigt worden, daß in nukleinsäurearmen Kernen während der Prophase eine Nukleinsäureanreicherung stattfindet. Diese vollzieht sich in den Chromosomenelementen. Um näher zu untersuchen, mit welchen Ereignissen in der Zelle die lokalisierte Synthese dieses eigenartigen hochpolymeren Stoffes verknüpft sein könnte, wurde mittels Ultraviolettabsorptionsmessung in der einzelnen lebenden Zelle die Gesamtmenge der Nukleinsäure während der verschiedenen Stadien der meiotischen Prophase bei *Gomphocerus* untersucht. Es stellte sich heraus, daß wenigstens vom mittleren Leptotän an die Nukleinsäuremenge konstant ist, was gegen eine direkte Korrelation zwischen Nukleinsäuresynthese und Chromatidenkontraktion spricht.

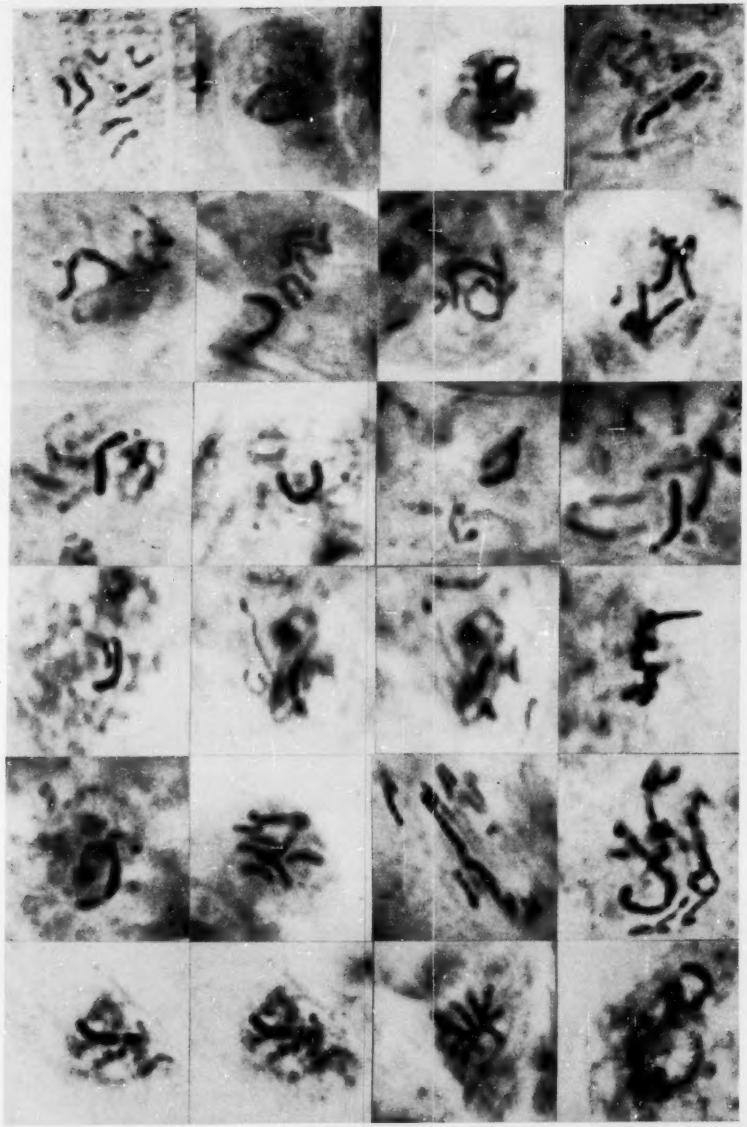
Da die Desoxyribosenukleinsäuren so elektiv in den gentragenden Elementen der Zelle lokalisiert sind und vor der Zellteilung in großen Mengen auftreten, zu der Zeit, in welcher vermutlich die Genreduplikation sich vollzieht, ist eine Korrelation zwischen diesen beiden Phänomenen als wahrscheinlich anzunehmen.

Literaturverzeichnis.

Astbury, W. T. and **Fl. Bell:** Nature (Lond.) **141**, 747 (1938). — **Belar, K.:** Roux' Arch. **118**, 359 (1929). — **Caspersson, T.:** Skand. Arch. Physiol. (Berl. u. Lpz.) Suppl. **73**. 1936. — **Caspersson, T.** and **J. Schultz:** Nature (Lond.) **142**, 294 (1938). — **Ekblom, T.:** Z. Zellforsch. **21**, 510 (1934). — **Signer, R., T. Caspersson** and **E. Hammarsten:** Nature (Lond.) **141**, 122 (1938).



EDGAR KNAPP und ILSE HOFFMANN, Geschlechtsumwandlung bei Sphaerocarpus.



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